THE UPTAKE AND LOSS OF ZINC AND LEAD BY *SCAPANIA UNDULATA* (L.) DUM., IN RELATION TO ITS USE AS A MONITOR

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ABSTRACT

Scapania undulata (L.) was studied in relation to its use as a monitor of zinc and lead pollution. An important characteristic of such a monitor is the sensitivity with which it mirrors change. To investigate this, clumps of the bryophyte were transplanted between sites which differed in their ambient metal concentration. A second lotic bryophyte, Chiloscyphus polyanthus (L.) Corda. var. rivularis (Schrad.) Nees., was also transplanted to to act as a comparison.

The enrichment ratios of zinc and lead were determined for both S. undulata and C. polyanthus var. rivularis. An enrichment ratio can be defined as: the factor by which an element is concentrated by biological accumulation. It is a particulary useful concept in monitoring studies. If the enrichment ratio remains relatively stable, the concentration of a metal in the monitor can be used to assess the past enviromental concentration.

The enrichment ratios displayed by S. undulata varied considerably, with an average coefficient of variance of approximately 60%. Three factors were thought to act as possible sources of variation:

i) some populations of S. undulata displayed an increase in the concentration of accumulated zinc and lead irrespective of the ambient concentration;

ii) the transplants indicated that the response to changes in the ambient metal concentration was not always rapid;

iii) the concentration of zinc and lead increased markedly down the bryophyte stem and therefore the metal concentration of a sample is influenced by the length of stem taken.

It would not be possible to use S. undulata accuratey as a monitor of ambient zinc and lead concentrations until the effects of these factors are understood.

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CHAPTER 1

INTRODUCTION

1.1 Monitors and indicators

The concept of biological indicators or monitors is not new: interest in them, in one form or another, has been of long standing. Cannon(1971) quotes the use of plants to locate water sources, during the pioneer phase of the American west. Since the turn of the century , this previously intuitive approach has been developed on a more scientific basis.

In order to be accepted as a potential indicator or monitor, the organism(s) must display a measurable characteristic which reflects the presence and, or level of, an environmental variable. The difference between an indicator and a monitor appears to be confused in the literature. For this project the following definitions are used. An indicator is a species, or community of species, which by its: presence, numbers, growth form or physiological state reveals the existence of a particular environmental factor(s). A monitor, by the same characteristics, reflects the variation in the level of a particular environmental variable(s).

The difference between a monitor and an indicator is not merely a question of semantics; the qualities demanded of the two are different. Odum (1959) describes indicators as 'steno' types which must have a restricted range of tolerance of variation in the environmental variable of interest. A monitor must in contrast tolerate a wide range of the variable monitored.

'Copper mosses' are adapted to growing on inorganic substrates such as rock surfaces(Shacklette, 1965). These plants, such as the liverwort <u>Gymnocolea acutiloba</u>, are restricted to, and therefore act as, indicators of copper deposits. The brown marine littoral algae, such as _Laminaria digitata_ and _Fucus vesiculosus_ (Young and Langille, 1958; Gutnecht, 1965; Foster, 1976) have been used as monitors of heavy metals in sea water. They have a widespread distribution throughout the U.K. coastal waters and occur even in estuaries which are highly contaminated by heavy metals.

1.2 Theoretical requisites of a monitor

The characteristic studied in a monitor must show a change which is proportional to that shown by the environmental variable of interest. This is often quoted in the literature (Dietz, 1973; Lloyd, 1977). However, before a monitor is introduced as a practical means of assessing the environment, the following questions must be answered.

i) Is the organism geographically widespread?

ii) Is a substantial range of values shown by the environmental variable capable of being 'recorded' by the monitor? What are its maximum and minimum effective limits?

iii) What is the relationship between the relevant characteristic of the monitor and the level of the particular environmental variable?

iv) How is the relationship determined in (iii) affected by changes in the environmental variable ?

v) How rapid is the monitors response to change?

vi) Is the relationship determined in (iii) consistent from individual to individual? Are there resistant races which, in the case of heavy metal monitors, might effect the rate of accumulation?

vii) Is the relationship determined in (iii) consistent in all environmental situations and at all times of the year?

1.3 Community monitors

The use of communities of species to monitor changes in the environment, particulary with regard to pollution, is widespread. This is based on the assumption that if community structure can be analysed any change in it can subsequently be determined. The methods used vary, but basically there are two types: those borrowed from the phytosociologists, which analyse the frequency at which species occur together; and those derived from the analysis of species diversity.

A review of the applications of the community approach is too large a subject to be included in the present study. Reviews can be found in: Hawkes (1979) on invertebrates, Sládeček (1979) on the European saprobic system, and Whitton (1979) on the use of algae. Recent work by Descy (1975) on diatoms and Empain (1978) on bryophytes illustrates the use of ordination analysis. The use of phytosociological groupings of vegetation with respect to geochemical prospecting is discussed by Cannon (1971).

1.4 Use of individual species as monitors

A single species has the advantage that its autecology can be more effectively studied, in contrast to the complex relationship of a community to its environment. As a consequence, the single species is popular for detailed monitoring studies. Hassibot (1975) described the use of captive fish to assess the mercury content of a discharge. Davis and Beckett(1978) used young plants to detect metal accumulation in soils which were fertilized with sewage sludge.

An important aspect of many studies of single species, used as monitors of heavy metal contamination, is the use of enrichment ratios. The term enrichment ratio was introduced by Brooks and Rumsby (1965); it forms a useful means of assessing the relationship between the metal concentrations in the surrounding medium andthose in the monitor in a quanititative manner (see Section 1.2 (iii)). It is defined by Whitton and Say (1975) as:

$$\frac{\text{concentration in organism (dry wieght)}}{\text{concentration in surrounding medium}}$$

Cyrptogams have shown themselves to be particularly useful in studies of heavy metal pollution (Margot and Romain, 1976). The concentration of metals accumulated by cryptogams were shown to be higher than those in higher plants. High enrichment ratios were found , for algae and bryophytes, by Dietz (1973), Say (1977), Lloyd (1977), and Harding (1978).

The stability of the relationship between the metal level 'recorded' by the monitor and that of the environment is an important characteristic of a monitor (Section 1.2(iii-vii)). This can be assessed, by determining the stability or otherwise of the enrichment ratio of a potential monitor, over a range of environmental conditions and seasons.

Keeney _et al_.(1976) used _Cladophora glomerata_ as a 'space/time' monitor for heavy metals in the Lake Ontario catchment. It displayed a narrow range of enrichment ratios: 1000-2900 for zinc and 16000-20000 for lead. Whitton (in press) reported a linear relationship between concentrations of zinc and lead in _Cladophora_ sp. and the concentrations in the surrounding water at the time of collections. The enrichment ratio for zinc was approximately 1300 over a range of zinc concentration in the water of 0.01-0.35 mg l^{-1}. Similarly, Gilva (1964) found enrichment ratios for zinc in _Fontinalis antipyretica_ to be constant over an ambient concentration range of 0.007-0.5 mg l^{-1} Zn.

It is generally accepted that bryophytes have among the highest enrichment ratios found for heavy metals (Kirchman and Lambinon, 1973; Dietz, 1973). Indeed, mosses possess remarkable ion exchange properties similar to those of many ion exchange resins (Climo, 1963). Apart frmm their high level of accumulation, aquatic bryophytes are usually common, and often form a large biomass which survives throughout the year. They absorb minerals directly from the water, having no effective roots or vascular tissue. In conclusion, bryophytes have been found to be ideal monitors for heavy metals.

Whitehead and Brooks (1970) used aquatic bryophytes in prospecting for uranium in the Buller Gorge region of New Zealand. They found concentrations of up to 100 µg g^{-1} uranium in bryophyte tissue.

Kirchmann and Lambinon (1973), and Kirchman et al.(1974) used bryophytes as part of a 'net of surveillance' set up to determine dispersion, within the environment, of nuclear effluent. A pressurised water reactor in the French Ardennes released effluent into the River Meuse. However, direct surveillance was difficult to maintain due to the low level of contamination. A general survey of the flora and fora revealed that bryophytes showed the highest and most consistent level of accumulation. A survey was subsequently undertaken using the moss, Cinclidotus danubicus, as a monitor. Over the three years studied, the concentration of radionuclicides in the moss peaked in the summers of 1969, 1970 and 1971. The concentration accumulated during the summer of 1971 were of an order of magnitude higher than those during the other two summers. This was as a consequence of a release of radioactive material, following work on the reactor,during the summer of 1971.

Empain (1974, 1976) studied heavy metal accumulation by bryophytes, in the River Sambre and River Meuse in Belgium,and the River Somme in France. He used four bryophyte species as monitors of the heavy metal load in the rivers. Accumulation of zinc and lead by three of these species was in reasonable agreement with the concentrations found in the water (Table 1).

Table 1. Relationship between concentrations of lead in river water and and that in three bryophyte species from River Meuse (Empain, 1976).

	location		
	Namur	Liege	
water	15	50	($\mu g\ l^{-1}$)
Rhynchostegium riparioides	100	300	($\mu g\ g^{-1}$)
Cinclidotus danubicus	50	300	($\mu g\ g^{-1}$)
Cinclidotus nigricans	130	300	($\mu g\ g^{-1}$)

Goodman and Roberts (1970) introduced the idea of using moss suspended in nylon bags to measure atmospheric pollution. This study was followed by Little and Martin (1974) who used Sphagnum sp. suspended in nylon bags to detect aerial pollution from a smelter in Avonmouth. The results showed a good correlation with the expected pattern of deposition as predicted from a wind rose.

Monitors do, therefore, produce results of practical relevance. They concentrate ambient metal levels, ideally showing a relatively stable enrichment ratio, so that any change in metal concentrations can be reflected by the monitor. It is also stated that monitors integrate variations in ambient metal concentrations (Empain, 1974; Foster, 1976). The processes upon which these statements are based, the uptake and loss of metals by the monitor, are, however, rarely investigated in any detail.

Harding and Whitton (in press) investigated the dynamics of zinc, lead and cadmium uptake and loss in Lamanea fluviatilis. They discovered that after transplanting the algae, between reaches where the zinc and lead concentrations of the water differed by an order of magnitude, the algal metal concentration took 1200 h or more to reach an asymptote. They demonstrated, however, that approximately 20% of the accumulated heavy metal concentration was in sensitive equilibrium with the medium and reflected rapidly any change in ambient metal levels.

Dunker (1974) followed the uptake of zinc by Scapania undulata to see if it was affected by other environmental factors. Temperature affected the final asymptote, but the initial rate of accumulation was dependent on the concentration of zinc in the water. Under laboratory conditions the absence of light reduced the final concentration by 15%.

1.5 Aims of the investigation

Scapania undulata has a wide geographical distribution, occurring in fast flowing upland streams and, under shade, in slow flowing lowland rivers (Watson, 1968). Say (1977) records it as having a wide distribution in upland streams, with a preference for water of low pH. During his study, in the North Pennine Orefield, Scapania undulata was found throughout the range from uncontaminated streams to highly polluted ones with up 7.0 mg l^{-1} zinc. Jones (1940), reporting on the River Ystwyth (wales), records Scapania sp. as the only macrophyte present at a zinc concentration of 1.2 mg l^{-1}. McLean and Jones (1975) found S. undulata in water with up to 6.9 mg l^{-1}. It was the tolerance to heavy metals of this species that led Benson-Evans and Williams (1976) to suggest its use as a monitor for mine effluent pollution studies.

The aim of this project was to investigate some characteristics of S. undulata, which affect its suitability as a monitor of zinc and lead contamination of rivers. An important aspect in these respect was the stability of the enrichment ratio displayed by S. undulata and how changes in ambient metal concentrations affect this (see Section 1.2 (iii - iv)). To investigate this clumps of the bryophyte were transplanted between reaches of a river which differed in their metal concentration. The response of S. undulata to transplantation was followed by repeated sampling of the bryophyte material (see Section 1.2 (v)).

CHAPTER 2

SITE AND REGIONAL DESCRIPTION

2.1 Introduction

The study was undertaken in the North Pennine Orefield, a region of historical importance for lead and zinc mining. Contamination from old workings, adits and from present day mining activity is, in places, serious (Harding, 1978).

The orefield covers approximately 3885 km^2 in the counties of Cumbria, Northumberland, Durham and West Yorkshire. Structurally it consists of the most northern of the three geological blocks which form the Pennines. It is dissected by three principle rivers of the North East, the Tyne, Wear and Tees, all draining eastwards. The combination of the Ice Age and fluvial action has produced rugged fell and dale scenery.

Heal, Jones and Whittaker (1975) describe the development of the present vegetation. Glacial drift covered the whole area after the last glacial retreat 13000 B.C. The Boreal/Atlantic transition at about 5500 B.C. was marked by an increase in rainfall. The boulder clay impeded drainage and the resultant waterlogging led to widespread peat accumulation. On the fells (510-888 m), *Sphagnum*, *Eriophorum*, and *Calluna* communities now dominate; these support only rough sheep grazing. Where the underlying strata are exposed, or where rivers have deposited alluvium, arable farming may exist, but pastural agriculture dominates.

2.2 Climate

The area is subject to cool damp conditions, with frequent cloud cover and high rainfall (annual mean of 190 cm). Temporary periods of low rainfall, leading to drought conditions, occur occasionally in the summer. The mean temperature for the coldest month, February, is 1.0°c, and the lowest minimum over a ten year period was

$-18.3^{o}C$. Snow cover is present for an average of 67 days per year between November and April, but the cover is irregular both in time and space. The climatic regime described is taken from Heal, Jones and Whittaker (1975) and refers to a meteorological station at the top of Weardale at 558 m.

2.3 Geology

The description following is based on Dunham (1948). The surface rock formation is Upper Carboniferous Limestone Series, consisting here mainly of beds of sandstone and shale of great thickness. This is underlain by Great Limestone and overlain by Millstone Grit Series, which outcrops on higher ground, forming caps to some of the fells. Surface formations are of a considerably higher horizon than the ore beds. Some oreshoots are,however, present as high as the Millstone Grit.

2.4 History of mining

Mining did not commence until the Norman conquest in the 11th century. For though there is much evidence of Roman presence in the area there is none to support mining activity. Extensive mining did not commence until 1650. Particularly important was the domination by Sir William Blackett and Family and the London Lead company. They controlled the area's mines during the period of greatest activity 1790-1882. Until 1896 all mining activity was for lead or silver; from 1896 to 1921 old mines were reworked for zinc. At present, only non-metallic resources are mined, mainly the gangue material fluorspar.

2.5 Experimental site

A stretch of the River Derwent and its tributary Bolts Burn were used for this study. The Derwent is a major tributary of the Tyne, situated in the most north-easterly area of the orefield. It is formed at 260 m by the confuence of Beldon and Nookton Burns; 1.5 km downstream of which, Bolts Burn joins the Derwent. The region surrounding Bolts Burn is one of the more intensely mined areas of the orefield. The valley is bordered by a large number of tailing heaps, disused mine buildings and adits (Say 1977). At the source of Bolts Burn there is an active fluorspar mine.

The Beldon, Nookton and Bolts Burns rise on the fells at an average height of about 510 m. The fells at this altitude consist of wide areas of peat moorland used for sheep grazing and grouse shooting. The Derwent falls 76 m in the 4.5 km from its source to the Derwent Reservoir, at which point it has a direct catchment of 721 ha.

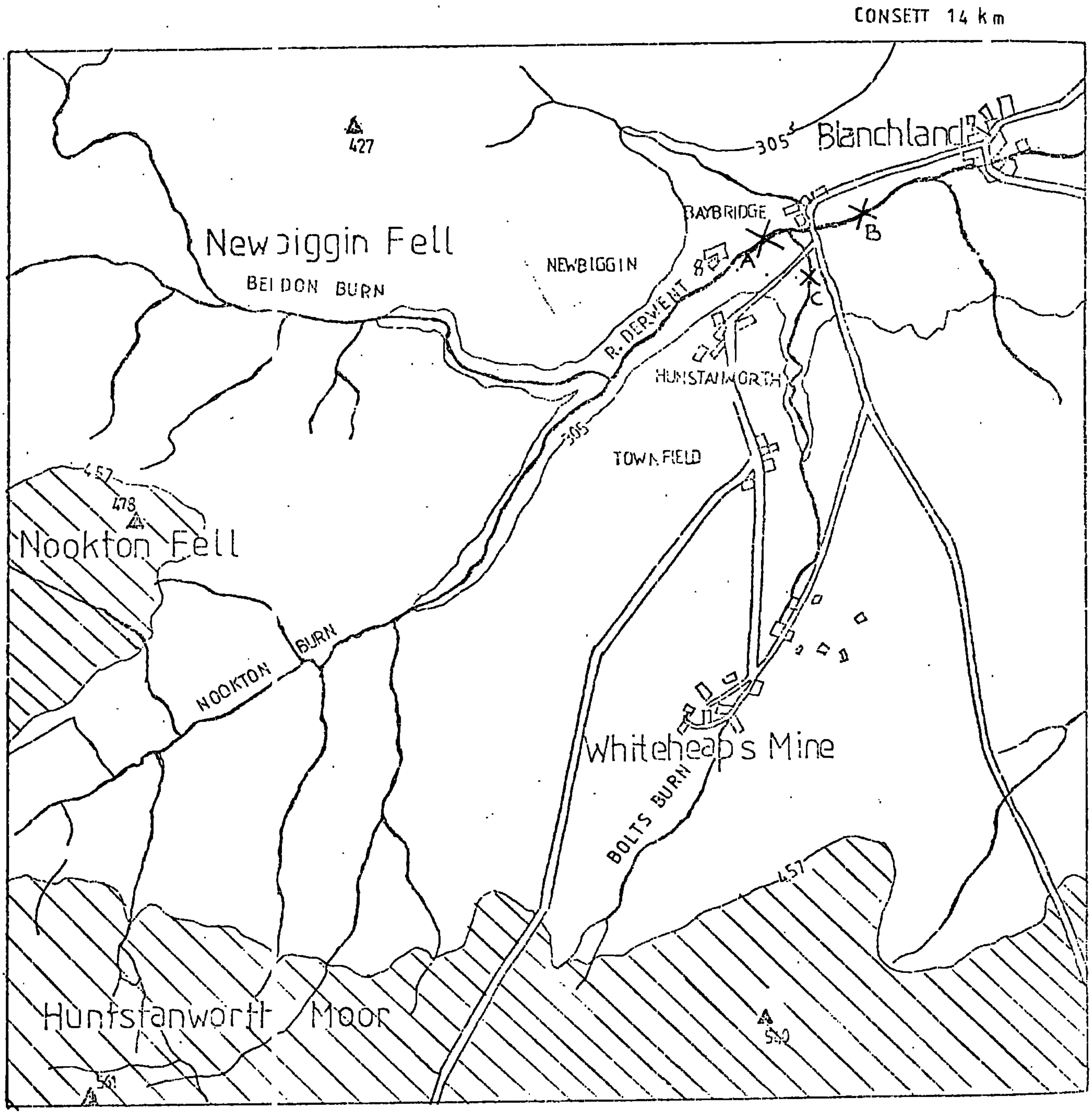
The Derwent carries downstream fairly high levels of brown humic material, derived from peat erosion. At its confluence with Bolts Burn it is a fast flowing but shallow river, with an average depth of 30 cm and a width of nine metres. Bolts Burn , in this area, is shallower, having an average depth of 12 cm and an average width of four metres. Both rivers fluctuate greatly in flow depending on weather conditions.

The population of the region is sparse. There are three small population centres in the vicinity : Baybridge, Newbiggin, and Hunstanworth. The latter discharges sewage from a small plant into the Derwent opening 0.5km upstream of the confluence with Bolts Burn.

2.6 Sources of zinc and lead contamination

The only mine in the area still active is Whiteheaps, at the head

Figure 1. Map of region surrounding transplant sites.



Scale 3·3cm = 1km

A reach 0061 05

B reach 0061 11

C reach 0071 98

of Bolts Burn. Originally a lead mine, it is now run by the British Steel Corporation for the production of fluorspar. Extraction commenced in 1924 and to date 125000 tonnes of fluorspar have been produced. Much of the gangue was too siliceous for early operations; however, a treatment works has recently been installed (Harding, 1978).

There are three distinct sources of contamination entering Bolts Burn:

i) erosion of old mine workings and tailings;

ii) an adit pump from the Whiteheaps mine;

iii) the fluorspar treatment works.

Runoff from the mine workings is of relatively minor importance. However, in periods of heavy rain it may be significant. The tips within the Whiteheaps mine area consist of fine particulate material and are easily eroded.

The adit discharge enters downstream of a tributary, Sikehead stream. The discharge is driven by an automatic pump whose activity is controlled by the level of the water collecting within the mine. Harding (1978) concluded that this was the single most important source of zinc and cadmium, while lead levels were relatively low. He recorded average levels of 6.617 mg l^{-1} zinc and 0.07 mg l^{-1} lead. This high zinc concentration was found to be relatively stable. The water is also characterised by high concentrations of Na, Mn and Fe.

The effluent from the treatment works enters 350 m downstream of the adit level. On most occasions this forms the most important source of particulate material and lead (Harding, 1978). Levels of 1.251 mg l^{-1} of lead were recorded.

The treatment involves the screening, crushing and separation by floatation of the gangue. The effluent from this process is a grey sludge composed of finely ground particulate material, mostly fluorspar and siliceous matter with a small amount of galena (lead sulphide).

The effluent passes through three settling ponds before discharge. Concentrated solutions of ferrous sulphate followed by sacks of powdered lime are added, which precipitates a blanket of ferric hydroxide. This sediments heavy metals by precipitation and adsorption.

The fine particulate matter colours Bolts Burn to its confluence with the Derwent. Deposits of material are sedimented throughout its length.

2.7 Transplant sites

The water of Bolts Burn, with its high levels of zinc and lead, is diluted at the confluence with the relatively uncontaminated waters of the Derwent. There are therefore, waters at three levels of heavy metal contamination within close proximity of each other. Three transplant sites were arranged so as to take advantage of this.

In accordance with a computer orientated recording system used for water, sediment and plant samples (Whitton, Diaz and Holmes, 1976); all rivers were identified by a four digit stream number. Bolts Burn was identified as '0071' and the River Derwent as '0061'. A reach was defined as being a 10 m long stretch where the volume and quality of the water entering and leaving was approximately the same. Each reach was allocated a two digit number in ascending order down stream. The three transplant sites were:

i) immediately above the confluence with Bolts Burn (reach identifier 0061 05, grid reference NY957498);

ii) 400 m below the confluence with Bolts Burn (reach identifier 0061 11, grid reference NY967504);

iii) Bolts Burn 20 m above the confluence with the River Derwent (reach identifier 0071 98, grid reference NY957498).

All transplant sites will, from here on, be refered to by their reach identifier.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction to methods

Two series of transplants were undertaken using Scapania undulata. The long term transplant (refered to as the L.T. Transplant) covered a period of 58 days. The sampling of S. undulata over this period would, it was hoped, reveal the rate at which the metal content of the bryophyte changed in response to its transplant. A short term transplant (S.T. Transplant) was done to investigate in detail the response of S. undulata in the 48 hours immediately following its transplant.

A second liverwort, Chiloscyphus polyanthus var. rivularis was transplanted between reaches 0061 05 and 0061 11. These acted as a comparision for the transplants using S. undulata, over the L.T. Transplant.

3.2 Taxonomy

Scapania undulata (L.) Dum. shows great morphological variation. Indeed Watson (1968) describes two extreme forms known originally as S. dentata (Dum.) and S. undulata (L.) now included within the one species. The former is reddish purple with toothed lobes, while the latter is green with entire lobes. The Scapania from the River Derwent is typically of the green and entire leaf lobe form. It has robust leafy shoots, erect or ascending with relatively few branches.

In its most robust form, S. undulata can form large tufts up to 15 cm in length (P.J. Say, personal communication). In the River Derwent growth is not as profuse, perhaps as a consequence of the rate of flow. Under trees in fast flowing stretches, clumps of about 4.0 cm in height cover boulders towards the bank. In less favourable sites a coating of about 0.5 cm in height is found.

Chiloscyphus cf. *polyanthus* (L.) Corda var. *rivularis* (Schrad.) is a highly branched pale green leafy liverwort. Being more flimsy and less robust than *S. undulata* it forms horizontal mats clinging to the surface of boulders or to other bryophytes. The species of *Chilosyphus* can be difficult to separate and it is possible that the species observed was *C. pallescens* (Ehrh.) Dum.

Both species used for the transplant experiments will, from here on, be refered to by their generic names.

3.3 Preliminary survey

An exploratory survey was undertaken (26-29 April)covering the lower reaches of Bolts Burn and the reaches of the River Derwent above and below the confluence with Bolts Burn. This covered the proposed transplant sites (2.7) and involved the study of the physico-chemical condition of the water and the metal content of the bryophyte flora.

3.4 Water chemistry

Any change in the environmental conditions could potentially affect the uptake or loss of zinc and lead by the bryophytes. In order to dertermine whether any such change did occur , over the period of study, a number of physical and chemical variables were regularly measured. Analysis of water chemistry was kept to a minimum as a detailed determination of water chemistry was undertaken in a project running concurrently (I.G. Burrows, M.Sc. Thesis in preparation).

The following data were collected: temperature, pH., optical density and a subjective estimate of flow. Temperature was measured using a mercury thermometer, while pH readings were obtained on a (P.Y.E) field pH meter(model 293) calibrated in the field with pH 7.0 and 4.0 buffers. Optical density (420 nm)was measured on a uvispect spectrophotometer in 40 mm cells,using a sample collected in a heavy duty polythene bottle directly from the main flow of the river. This sample was stored in an ice box until the return to the laboratory.

3.5 Methodology

3.51 Zinc and lead determination

All equipment which was involved in zinc and lead analysis was acid washed, to remove possible sources of contamination. This entailed it being soaked in 10% hydrochloric acid (HCl) for half an hour and then rinsed six times in single distilled water.

The concentration of zinc and lead in the river water was measured whenever samples of bryophyte material were taken. A large volume of water was collected in an acid washed bucket. This was left to stand for 5 minutes, to allow the sedimentation of the larger particles. Two water samples were subsequently taken; a 'filtered' sample and a 'total' sample. The former was passed through a 0.2 µm Nuclepore polycarbonate membrane filter held in an acid washed swinex filter holder. The filters were washed in distilled water and not acid washed. Acid washing of nuclepore filters is suspected to increase their cation exchange capacity (Harding, 1978). An acid washed 15 ml syringe was used to force the river water through the filter. The first 5 ml to pass through the filter was discarded, the rest was added to an 8 dram glass vial (acid washed); a total of 25 ml was collected. The 'total' sample was obtained by pouring approximately 25 ml into another vial from the bucket.

One drop of atomic absorption grade nitric acid (A.A.G. HNO_3) was added to all water samples collected for metal analysis. The addition was carried out to lower the pH below 1.0 to minimise microbial activity, prevent precipitation and to increase the proportion of ionic species present.

Zinc and lead concentrations were determined using a Perkin Elmer 403 atomic absorption spectrophotometer. Aspiration with an acid resistant nebuliser was used for zinc and high levels of lead. The Tm. sampling boat procedure (Kahn <u>et al</u>., 1968) was used for low lead concentrations.

3.52 Collection of plant material

Samples of bryophyte material consisted of a series of sub samples which were taken from different clumps of liverwort. The material was washed in river water to remove loose sediment and then put into a plastic bag. All bags were washed in river water before use. The samples were stored in an ice box until such a time when fractionation (section following) was undertaken.

3.53 Fractionation of plant material

Two divisions of the bryophyte stems were collected, the first two 1 cm lengths from the tip (these will be refered to as the 1 cm and 1-2cm fractions respectively). For Scapania the first 1cm fraction consisted of new and old foliate growth while the 1-2cm fraction consisted of stem and dead leaf material. It was thought that the dynamics of heavy metal uptake and loss might differ between the two fractions. With Chiloscyphus these differences between fractions were less clear. Chiloscyphus is more branched and faster growing than Scapania, so that later in the season the 1-2cm fraction included new growth.

Initially Chiloscyphus and Scapania were separated before fractionation. Later in the season however, it became possible to collect both separately.

Before fractionation, the material was again washed in river water. River water for washing was collected at the same time as the liverworts in a pre-washed plastic bag. The plants were then fractionated using a scalpel and forceps in a series of acid washed petri dishes. The fractions were then given another river water wash and finally a distilled water wash, after which they were dropped onto a paper towel to remove surplus moisture and each fraction was put into a glass vial. If fractionation was carried out in the field, the samples were returned to the ice box to be dried on return to the laboratory at 104°C for 48 hours. Plant material was stored in this form until digestion.

3.54 Digestion and Analysis

Before digestion, samples were redried for 24 hours and subsequently put into a desiccator to cool. After half an hour, samples were removed and immediately weighed (approximately 20mg weighed to 4 decimal places/fraction). Samples are capable of absorbing up to a third of their weight in moisture, so they were exposed to the air for as short a time as possible.

A sample when ready for digestion was emptied into an acid washed (100ml) Kjeldhahl flask. 3ml of A.A.G. HNO_3 was added to the now emptied vials to dissolve any remnants of sample remaining. After 10 minutes this acid was emptied into the Kjeldhahl flask. The acid was then boiled slowly for 30 minutes on an electric heating rack. The addition of distilled water after this period diluted and cooled the acid and the digest was poured into an acid washed 25ml volumetric flask. Two distilled water washes of the Kjeldhahl flask were added and the volumetric flask was finally made up to volume with distilled water. The digest was stored in its original vial at $4^{o}C$.

Zinc and lead concentrations were determined with a Perkin-Elmer 403 atomic absorption spectrophotometer, using aspiration for zinc and aspiration plus the boat method for lead, depending on the concentration range of the sample.

3.6 Long Term Transplant (L.T. Transplant)

Three transplants were undertaken, a reciprocal one between 0061 05 and 0061 11 and one from 0061 05 to 0071 98. This involved therefore, transplants from low to medium and high contamination and one from medium to low contamination. It was hoped that a 58 day period would allow the transplanted material to reach the level of lead and zinc found in the natural reach populations. To ascertain the progress of this process, samples were taken from both transplants and natural reach populations(controls). The sampling periods were arranged so as to concentrate on the early stages, when any change was thought to be the most rapid. Five replicate samples were collected from the transplants. Five replicate samples were collected for the controls only at the beginning and end of the experiment, for the other sampling dates only two were taken.

3.61 Movement of material

Fifteen medium sized bryophyte covered boulders were collected for each transplant. It was attempted to collect boulders with bryophyte populations of a similar morphological type. Even so, limitation in the resource did result in some clump variability.

The boulders were transported in a bucket full of water and deposited in what appeared to be similar microhabitats. All were placed in the shade of trees, in medium flow conditions, about 10cm below the water surface. This standardization, it was hoped, would isolate water chemistry as being the major difference between the sites. Another factor in the choice of sites was the presence of a natural reach population to act as a control.

3.62 Fractionation details

The time between collecting, fractionation and subsequent drying should be kept to a minimum. Variability is thought to be introduced by leaching, adsorption or bacterial/algal growth during this period. When possible therefore, fractionation was undertaken in the field, particularly when the results would be especially sensitive to error. During the first three sample periods fractionation was conducted in the field.

Fractionation took five to seven hours, so even allowing maximum efficiency the final sample will have been out of the water for more than five hours. The routine was standardised so as ,hopefully, to isolate any variability introduced. Reach 0061 05 material was dealt with first then 0061 11 and finally 0071 98.

3.63 Chiloscyphus transplant

Chiloscyphus was only sampled in the two River Derwent transplants. It therefore involved a transplant from low to high and one from high to low contamination.

The Chiloscyphus experiment developed by taking advantage of its presence in trace quantities during the initial stages of the transplant of Scapania. Originally it was sampled merely to act as a few spot samples for comparison with Scapania. As,however,its abundance increased due to its rapid growth, progressively more samples were taken. By day 15,full sets of five replicates were taken. Due to the nature of the development of this transplant no control samples were taken until the final sampling date, when a replicate of five was taken.

3.7 Short Term Transplants (S.T.Transplants)

This experiment involved the use of Scapania only. The results obtained from the L.T.Transplant were,it was felt, in need of clarification over the initial 48 hours. A more detailed study was therefore undertaken of this period. The sampling periods were arranged so as to be concentrated in the first 24 hours.

A replicate of five samples was taken for both fractions at the beginning and end of the transplant. No samples were taken,however, of the 1-2 cm fraction between, as the L.T. Transplant showed no change in this fraction over a period of three days. A single 1 cm fraction sample was taken every sample period from the transplant and the natural reach material (controls).

Only two transplants were carried out, a reciprocal transplant was undertaken between reaches 0061 05 and 0061 11 of the River Derwent. Movement was between the same sites as used for L.T. Transplants.

Due to the compressed nature of the sampling periods only temperature, pH, and total water samples were taken. A filtered sample was taken at the beginning and the end to illustrate the proportion that was particulate.

The procedure for sampling and fractionation was as described for the L.T. Transplant (3.42) with a few modifications. The samples were initially washed in river water and then in distilled water. They were then put in a plastic bag and stored in an ice box. All fractionation was undertaken in the field between sampling periods.

3.8 Growth experiment

It is important in order to interpretate the results of the L.T. Transplant to know the growth rate. To assess whether the 1 cm tip taken at the final sample is new growth or original material that was exposed to the pre-transplant conditions.

Three transplants were arranged as follows:

(i)	0061 05	⇒	0061 11
(ii)	0061 11	⇒	0061 05
(iii)	0061 05	⇒	0071 98

One large boulder with a good bryophyte population was moved, as above, to the same sites as used for L. T. Transplant. For each of the transplants and for one control in each reach, 60 <u>Scapania</u> stems were delimited 0.5 cm from the tip, with a piece of cotton. Half of these were harvested after 24 days and the rest after 51 days. Any change in length was determined.

3.9 Manipulation of results

The Northumbrian Universities Multiple Access Computers (NUMAC) were used for the data processing. The system is based on a IBM 360 and 370 main frames, running under the Michigan Terminal System. All data for plant heavy metal analysis was punched onto computer cards and stored on computer files. Two separate FORTRAN programs were designed, the first to convert weight and atomic absorption spectophotometer readings into $\mu g\ g^{-1}$ and the second to work out means, standard deviations and coefficiencies of variance. All graphs apart from the ratio diagrams were produced by the GHOST graphical output system (Eadie and Hall, 1977).

It should be noted that the standard deviations quoted in the text are of dubious value, as they have been calculated on the basis of only five replicates. They are used solely to give a rough indication of the variation of the values around the mean.

3.91 Ratio diagrams

A new technique devised to aid in the analysis of transplant experiment results was introduced. Ratio diagrams plot the proportion of a metal held relative to another metal. In this report the ratio diagrams were constructed by calculating the percentage of the total zinc and lead concentration which was held as lead:

$$Pb / (Pb + Zn). 100 = \text{level of percentage lead (L.P.L.)}$$

The aim of this technique is to facilitate the study of accumulation or loss of different metals by a monitor. The assumption is made that the rate of accumulation and loss of a metal is at least partially dependent on the concentration of that metal in the water.

If a comparision is to be made between the rates of accumulation or loss of zinc and lead, then the relative concentrations of these metals in the water must be taken into account. Ratio diagrams allow the relative metal concentrations of the water to be directly compared to thoseof the bryophyte, in terms of their L.P.L. . If the bryophytes do not show a tendency to reflect the L.P.L. of the water following transplantation, then some other factor(s) are influencing the accumulation or loss of the metals. One such fact could be an intrinsic difference in the rates of accumulation or loss of zinc and lead specific to the particular monitor.

Ratio diagrams were drawn to compare the effect of zinc and lead accumulation and loss (following transplantation) in Scapania and Chiloscyphus. The L.P.L. was calculated for the transplants, controls and the water in each reach.

CHAPTER 4

RESULTS

4.1 Preliminary survey

The preliminary survey (26 April - 1 May) revealed the presence of five bryophyte species (Table 2).

Table 2. Species list of bryophytes found in reaches: 0061 05, 0061 11 and 0071 98.

Scapania undulata (L.) Dum.

Rhynchostegium riparioides (Hedw.) Jenns.

Hygrohypnum luridum (Hedw.) Jenns.

Fontinalis antipyretica (Hedw.)

Chiloscyphus polyanthus (L.)Corda. var. *rivularis* (Schrad.)Nees

F. antipyretica was found only in reach 0061 05, above Bolts Burn. The other bryophytes, with the exception of *C. polyanthus var. rivularis*, were found to be common in reaches 0061 05 and 0061 11, but rare in reach 0071 98. *C. polyanthus var. rivularis* was rare in all reaches.

Table 3. Zinc and lead concentrations in the bryophytes in the River Derwent ($\mu g\ g^{-1}$), from the preliminary survey (26April to 1 May).

	1 cm	1-2cm	1 cm	1-2cm
	zinc ($\mu g\ g^{-1}$)		lead ($\mu g\ g^{-1}$)	
reach 0061 05				
S. undulata	255	446		
R. riparioides	406	1328		
H. luridum	350	525		
F. antipyretica	177	223		
reach 0061 11				
S. undulata	590	766	1218	2163
H. luridum	1623	2419		

The concentration of zinc in the bryophytes (Table 3) appeared to be related to the amount of new growth present. F. antipyretica, which consisted solely of new growth , had the lowest zinc concentration. R. riparioides and H luridum had high levels of zinc and displayed very little new growth. S. undulata was intermediate, both in terms of metal concentration and the amount of new growth.

4.2 Changes observed over the period of study

Scapania was found to be at its height of abundance and luxuriance during the spring. By May Scapania was common along the sides of the river, especially under the shade of trees; however, subsequently its abundance decreased.

Chiloscyphus was rare initially, being found in small quantities among clumps of Scapania. During May Chiloscyphus displayed a rapid rate of growth, so that by the end of the month it dominated some clumps of Scapania. By June large luxuriant clumps of pure Chiloscyphus covered boulders in reaches 0061 05 and 0061 11. In reach 0071 98 Chiloscyphus remained rare.

Bryophyte abundance decreased over the period of study in Bolts Burn as a consequence of a massive algal bloom. In April the bed of Bolts Burn was turned green by the growth of Hormidium spp. and from June this alga dominated the system. This phenomenon had not been noted in previous years and was possibly associated with the introduction, this year, of a polyelectrolyte to the treatment procedure at Whiteheaps mine (I. G. Burrows, personal communication). The bloom collected colloidal material from the water and formed a grey smothering mat on the stream bed.

The bryophytes in Bolts Burn were covered by a thick coat of slime, algae and detritus and by the end of June some clumps were partially moribund. Where Scapania was coated, new stems of a different growth form pierced through. These were not as robust, but were thin stemmed, with thin and delicate reduced leaves.

In the River Derwent, during June, Scapania and R. riparioides were coated by a mucilaginous layer of diatoms. This was particularly evident above the confluence with Bolts Burn in well illuminated stretches, where the abundance of Scapania was reduced. Bryophyte clumps under shade were relatively unaffected.

4.3 Physico-chemical conditions

There was over the period of the L.T. Transplant a gradual change in the environmental conditions (Table 10). The rate of flow decreased and associated with this the optical density of the water decreased. The water temperature and pH did not, however, show any consistent trend though the water temperature tended to be higherin the later stages of the transplant. During the S.T. Transplant the temperature and pH of the water was monitored over the 48 hours (Figures 15 and 16). This revealed the extent of the diurnal variation in water temperature and pH; a maximum of 5^{o}C and 1 unit of pH. A proportion of the variation in these variables found during the L.T. Transplant, could be a consequence of the fact that the samples were taken at different times of the day.

The concentrations of zinc and lead in the water peaked at Day 7 and Day 1 repectively (Figures 2 and 3). Concentrations fell from this maximum over the rest of the study period, though a recovery occurred in the zinc concentration of reach 0061 11 after Day 30.

Information on the level of other metals, fluride and phosphate was provided by I.G. Burrows (Appendix A). All metals, with the exception of iron, have higher concentrations in Bolts Burn than in the River Derwent. Fluride levels increase in the River Derwent below its confluence with Bolts Burn while the phosphate concentrations decrease.

4.4 Long term transplants

All transplanted bryophyte material displayed a change in accumulated metal levels. As a consequence of this change, the difference between the metal concentration of the transplants and the controls decreased. The rate at which this occurred varied from transplant to transplant.

4.41 0061 11 – 0061 05

At the end of the transplant experiment, Day 58, both the zinc and lead concentrations of the transplant material had reached similar levels to those of the controls (Figure 4 and 5). The difference was greatest in lead concentrations; the transplant material being 200 $\mu g\ g^{-1}$ higher in the 1cm fraction and 800 $\mu g\ g^{-1}$ in the 1-2cm fraction. To put this in perspective the difference between the transplants and the controls on Day 0 was 1400 $\mu g\ g^{-1}$Pb (1cm fraction) and 4300 $\mu g\ g^{-1}$Pb (1-2cm fraction).

The concentration of zinc and lead in the water fell in reach 0061 05 (Figures 2 and 3). This was reflected by a fall in the lead concentration of the controls over the period studied. The zinc concentration of the controls increased, however, over the same period (Table 4).

Table 4. Concentration of zinc and lead in the controls (*Scapania*) at the beginning and end of the transplant experiment (reach 0061 05).

		Day 0	Day 58
zinc ($\mu g\ g^{-1}$)			
	1cm	248 ± 38	400 ± 34
	1-2cm	467 ± 72	952 ± 151
lead ($\mu g\ g^{-1}$)			
	1cm	193 ± 8	128 ± 9
	1-2cm	456	249 ± 58

4.42 <u>0061 05 – 0061 11</u>

Both zinc and lead uptake showed no signs of reaching an asymptote, nor did they reach the level of accumulation shown by the controls(Figures 6 and 7). In reach 0061 11 the zinc concentration of the water increased, while the lead concentration fell slightly(Figures 2 and 3). The concentration of these metals in the controls increased dramatically (Table 5).

<u>Table 5.</u> Concentration of zinc and lead in the controls(*Scapania*) at the beginning and end of the transplant experiment (reach 0061 11)

		Day 0	Day 58
zinc ($\mu g\ g^{-1}$)			
	1cm	1674 $\pm$ 559	8561 $\pm$ 1232
	1-2cm	3659 $\pm$ 1708	24429 $\pm$ 4158
lead ($\mu g\ g^{-1}$)			
	1cm	1678 $\pm$ 297	5583 $\pm$ 1478
	1-2cm	4721 $\pm$ 609	9143 $\pm$ 428

4.43 <u>0061 05 – 0061 98</u>

Zinc levels rose very rapidly in the transplanted material overshooting the concentration shown by the controls and reaching a maximum of 13606 $\pm$ 7698 $\mu g\ g^{-1}$ at Day 15. After this peak the concentration of the 1cm fraction appeared to converge towards the level shown by the controls. The 1-2cm fraction remained at the higher level. The lead concentration of the transplants initially rapidly increased, levelling off just below that shown by the controls.

The metal concentration of the controls remained relatively stable in this reach (Figures 8 and 9). The 1cm fraction did show an increase in zinc until Day 3·, after which it levelled out.

4.44 Chiloscyphus transplants

The bryophyte material moved in this two way transplant between reaches 0061 05 and 0061 11, reached similar zinc and lead concentrations to those of the appropriate controls by Day 58 (Figures 10 and 11). The material transplanted into reach 0061 11, however, showed no sign ofapproaching an asymptote, indeed the rate of increase of zinc would appear to have been increasing.

The controls did not reflect the change in the zinc and lead concentration in the water over the study period. The zinc concentration in the water of reach 0061 11 increased by about two fold over this period (Figure 2). The concentration of this metal in the controls increased, however, by approximately seven fold (1cm fraction) and ten fold (1-2cm fraction). In the other cases the concentration of zinc and lead in the water either fell or remained at a similar level. The concentration of these metals in the relevant controls increased (Table 6).

Table 6. Concentration of zinc and lead in the controls(Chiloscyphus) at the beginning and end of the transplant experiments(reaches 0061 05 and 0061 11).

	reach 0061 05		reach 0061 11	
	Day 0	Day 58	Day 0	Day 58
zinc ($\mu g\ g^{-1}$)				
1cm	87	324	929	6425
1-2cm	350	788	2246	11947
lead ($\mu g\ g^{-1}$)				
1cm	120	254	2256	3029
1-2cm	319	663	5719	4615

4.5 Ratio diagrams

The ratio diagrams for the water at reach 0061 11, showed that the level of percentage lead (L.P.L., see Section 3.91) fell from 50%, at the start of the transplant experiment, to 10% by Day 7; where it remained for the rest of the study period. The available data suggests that a similar pattern exists in reach 0071 98. For reach 0061 05, the L.P.L. in the water fell slowly to approximately 15-25% by Day 30; followed by a recovery to 30-40% by Day 58.(Figures 12 and 13).

The ratio diagrams for the bryophyte material transplanted into water with a high metal concentration (reaches 0061 11 and 0071 98) were very similar (Figures 12, 13 and 14). The L.P.L. in Scapania and Chiloscyphus fell rapidly from approximately the 50%-70% range to 25%-10%. This was followed by a slow recovery in the L.P.L. to approximately 30%-50%. The L.P.L. in the control material from reaches 0061 11 and 0071 98 decreased slowly to 30%-50% by Day 58.

The bryophytes transplanted to, and the controls for, reach 0061 05 had similar ratio diagrams (Figures 12 and 14). There was a slow fall in the L.P.L. throughout the period of study.

4.6 Physico-chemical conditions during the short term transplant

The temperature of the river water at both reaches 0061 05 and 0061 11 varied in a cyclic manner over 24h, tending to reach a maximum between 16.00 and 18.00h (Figure 15). The pH of the water remained relatively stable; variation over a 24h period did, however, occur with a peak occurring a couple of hours earlier than the temperature maximum (Figure 16). Both effects are related to the amount of solar radiation, via direct heating of the water and the photosynthetic activity of the aquatic plants. Zinc and lead concentrations at reach 0061 11 also showed a diel pattern of variation (Figure 17). Zinc reached a maximum which coincided with the minimums of pH and temperature. The lead concentrations, however, peaked at the time of maximum temperature and pH. The lead concentrations at reach 0061 05 tended to vary erractically but suggest a similar pattern (Figure 18).

4.7 Short term transplant

The concentration of zinc and lead, in the 1cm fraction of Scapania, changed relatively rapidly following transplantation (Figures 19 and 20). Over the 2days, the rate of loss and accumulation of zinc and lead, was greater than that observed over the initial phase (Day 0 to Day3) of the L.T. Transplant (Table 7). This difference was particulary pronounced in the case of the rate of loss (transplant 0061 11 006105), which, in the S.T. Transplant, exceeded the rate of accumulation (transplant 0061 05 0061 11).

Table 7. Comparison of the rates of accumulation and loss of zinc and lead, by the 1cm fraction of Scapania, in the L.T.Transplant and the S.T.Transplant. Figures calculated from; the change in metal concentrations over the first three days of the L.T.Transplant, and the change in metal concentrations overthe two days of the S.T. Transplant.

	0061 11 – 0061 05		0061 05 – 0061 11	
	zinc	lead	zinc	lead
L.T.Transplant rate in $\mu g\ g^{-1} d^{-1}$	– 20	no decrease	+ 180	+ 100
S.T.Transplant rate in $\mu g\ g^{-1} d^{-1}$	– 2300	– 700	+ 1110	+ 440

The 1-2cm fraction did not show any decrease in zinc or lead concentration, when transplanted from reach 0061 11 to 0061 05. The zinc and lead concentration of this fraction did, however, increase over the two days when transplanted from reach 0061 05 to 0061 11.

4.8 Ratio diagrams and stort term transplant

The values for the zinc and lead concentrations in bryophyte tissue were obtained from single samples for the S.T. Transplant. This contrasts with the values obtained for the L.T. Transplant, which were derived from five replicate samples. A consequence of this was that the L.P.L. values for the S.T. Transplants show more, seemingly random, variation (Table 35).

Similarities with the results of the L.T. Transplants can be found, if the average of the L.P.L. values, over the period of the S.T. Transplant are examined (Table 8).

Table 8. Average L.P.L. values over the period of the S.T. Transplant for 1cm fractions of *Scapania undulata*.

	mean L.P.L. values
water; reach 0061 05	7 ± 2
controls; reach 0061 05	25 ± 6
transplant from 0061 05 to 0061 11	20 ± 4
controls; reach 0061 11	36 ± 4
transplant from 0061 11 to 0061 05	35 ± 3

In the L.T. Transplant, when material was transplanted from reach 0061 05 to 0061 11, the L.P.L. fell from approximately 60% to 20%-30%. by Day 15 (Figure 12). During the S.T. Transplant the same transplant was undertaken. The control material at reach 0061 05 had an average L.P.L. of 25%. When this material was transplanted from this reach to reach 0061 11, the average fell to 20%. In the opposite transplant from reach 0061 11 to 0061 05, the L.P.L. in the transplanted material was 35% very similar to that of the control material for reach 0061 11. This is consistant with the L.T. Transplant results, where for the transplant from reach 0061 11 to 0061 05, little change in the L.P.L. occurred in the first three days.

4.9 Growth rate

The growth rate of Scapania was determined over the period from the 22May to 19 July (Table 9). Small differences in the rate of growth were detected. As might be expected, the rate of growth was inversely correlated with the zinc and lead content of the water. All values had however overlapping standard deviations.

Table 9. Rate of growth of Scapania undulata from 29 May to 19 July (expressed as mm d^{-1}).

	0061 05	0061 11	0071 98
control populations			
mean	0.06	0.05	0.05
max.	0.12	0.1	0.1
S.D.	0.04	0.03	0.03
transplant populations			
mean	0.08	0.05	0.03
max.	0.18	0.14	0.08
S.D.	0.05	0.06	0.02

Table 10. Physico-chemical data collected during the long term transplant.

	O.D. (1 cm path)	pH	Flow (1-10)	T(°C)	Time(h)
22.05.79					
0061 05	0.106	7.44	7	9.3	9.00
0061 11	0.083	7.64	7	9.9	
0071 98	0.043	8.1		11.0	
23.05.79					
0061 05	0.183	7.3	10	10.0	14.00
0061 11	0.197	7.8	10	9.8	
0071 98	0.132	8.0		9.8	
25.05.79					
0061 05	0.049	7.5	6	7.2	10.00
0061 11	0.041	8.0	6	7.4	
0071 98	0.010	8.2		9.0	
29.05.79					
0061 05	0.144	6.9	7	11.0	16.00
0061 11	0.120	6.9	7	11.8	
0071 98	0.031	7.7		12.1	
06.06.79					
0061 05	0.020	7.2	5	11.2	14.00
0061 11	0.021	7.3	5	11.2	
0071 98	0.009	7.9		11.2	
22.06.79					
0061 05	0.021	7.8	2	10.0	12.00
0061 11	0.020	7.9	2	10.0	
0071 98	0.008	8.2		10.5	
19.07.79					
0061 05	0.019	7.5	1	10.0	13.00
0061 11	0.019	7.6	1	10.5	
0071 98	0.025	8.0		10.0	

<u>Figure 2.</u> Concentration of zinc in the water (mg l^{-1}), expressed as 'filtered' and 'total' concentrations, over the long term transplant (22 May - 19 July).

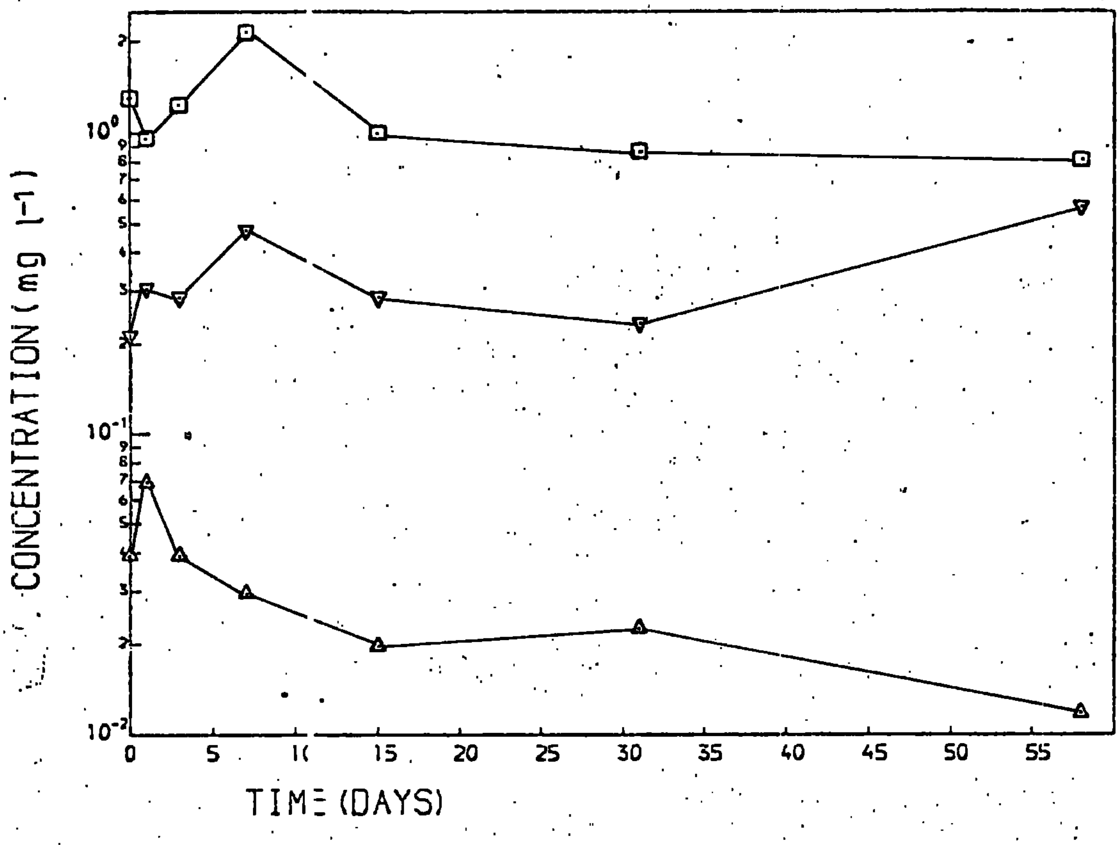
△ reach 0061 05

▽ reach 0061 11

□ reach 0071 98

See Table 23 for data (Appendix B).

"TOTAL" ZINC



"FILTERED" ZINC

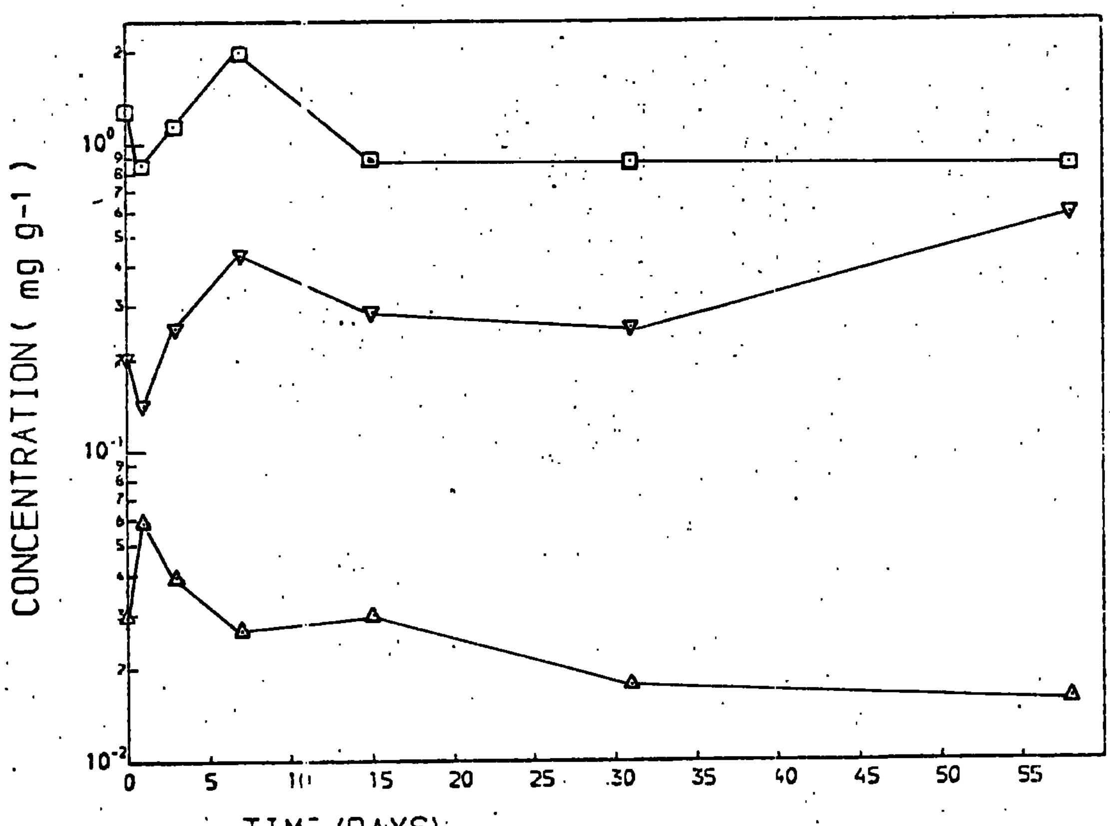


Figure 3. Concentration of lead in the water (mg l^{-1}), expressed as 'filtered' and 'total' concentrations, over the long term transplant (22 May - 19 July).

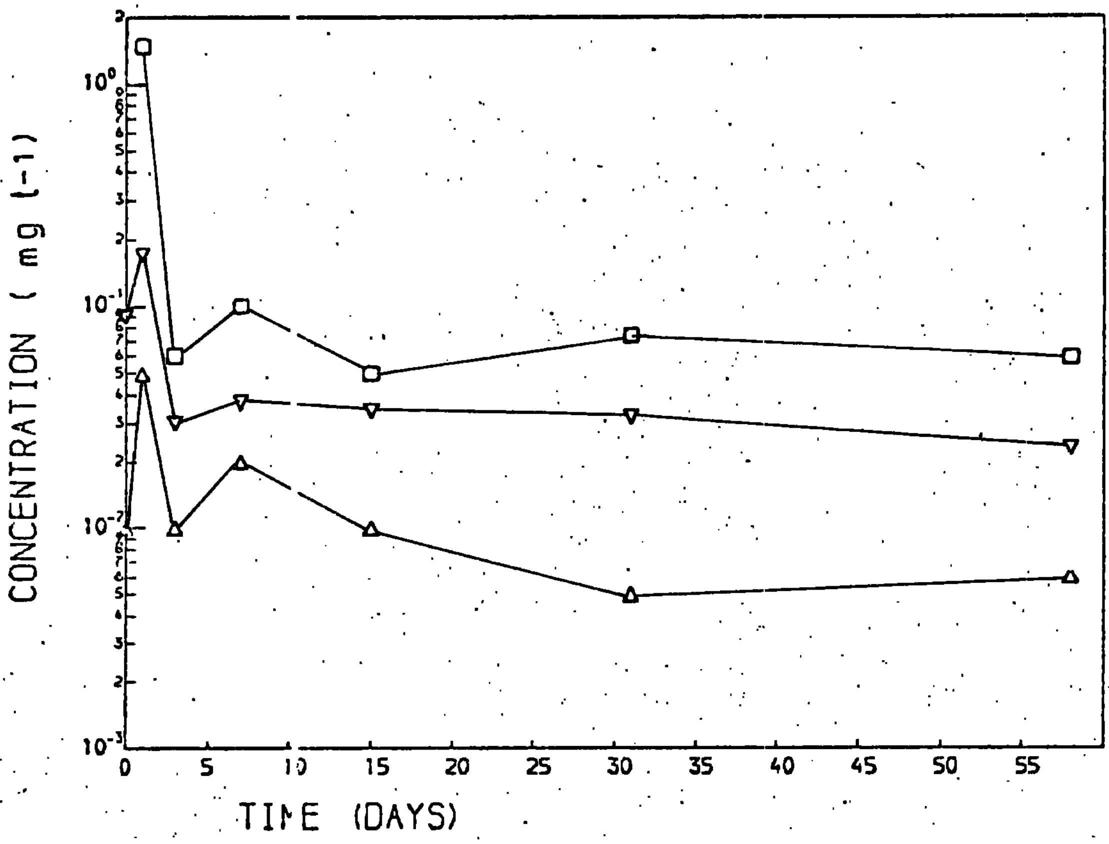
△ reach 0061 05

▽ reach 0061 11

□ reach 0071 98

See Table 24 for data (Appendix B).

"TOTAL" LEAD



"FILTERED" LEAD

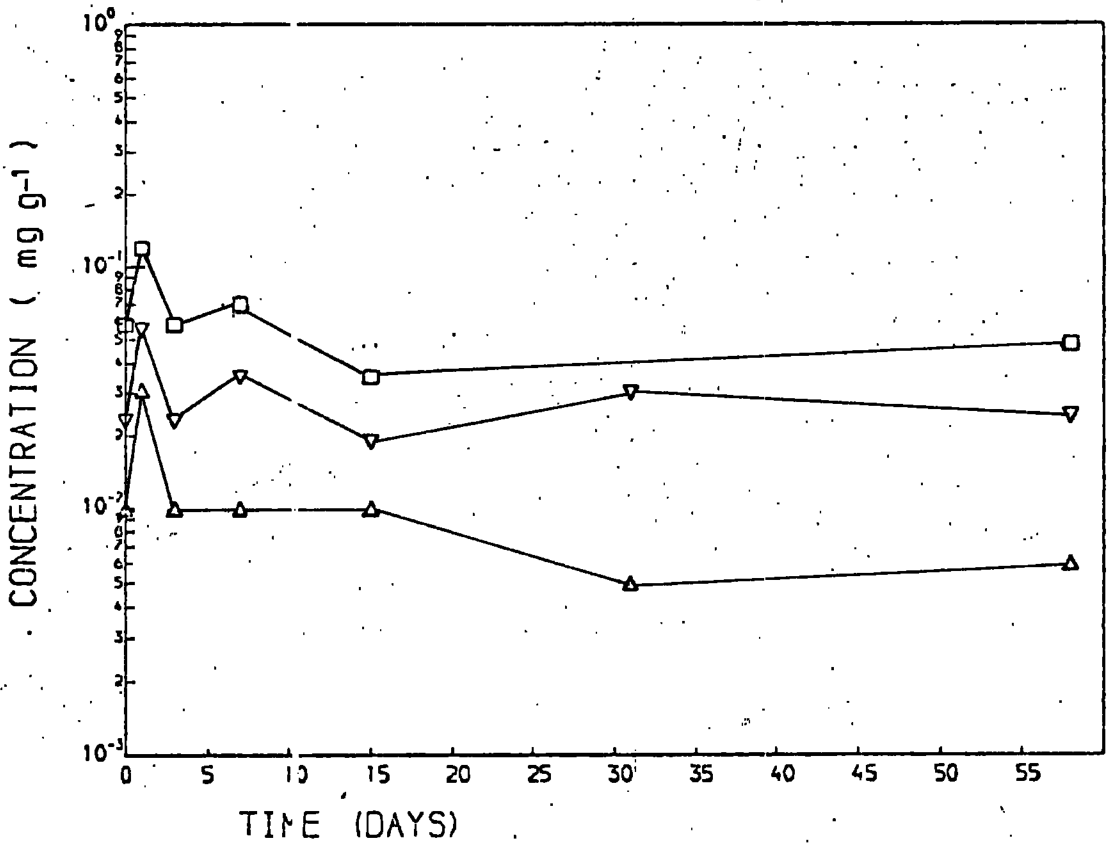


Figure 4. Concentration of zinc in Scapania undulata ($\mu g\ g^{-1}$) as observed in reach 0061 05 during the long term transplant (22 May - 19 July).

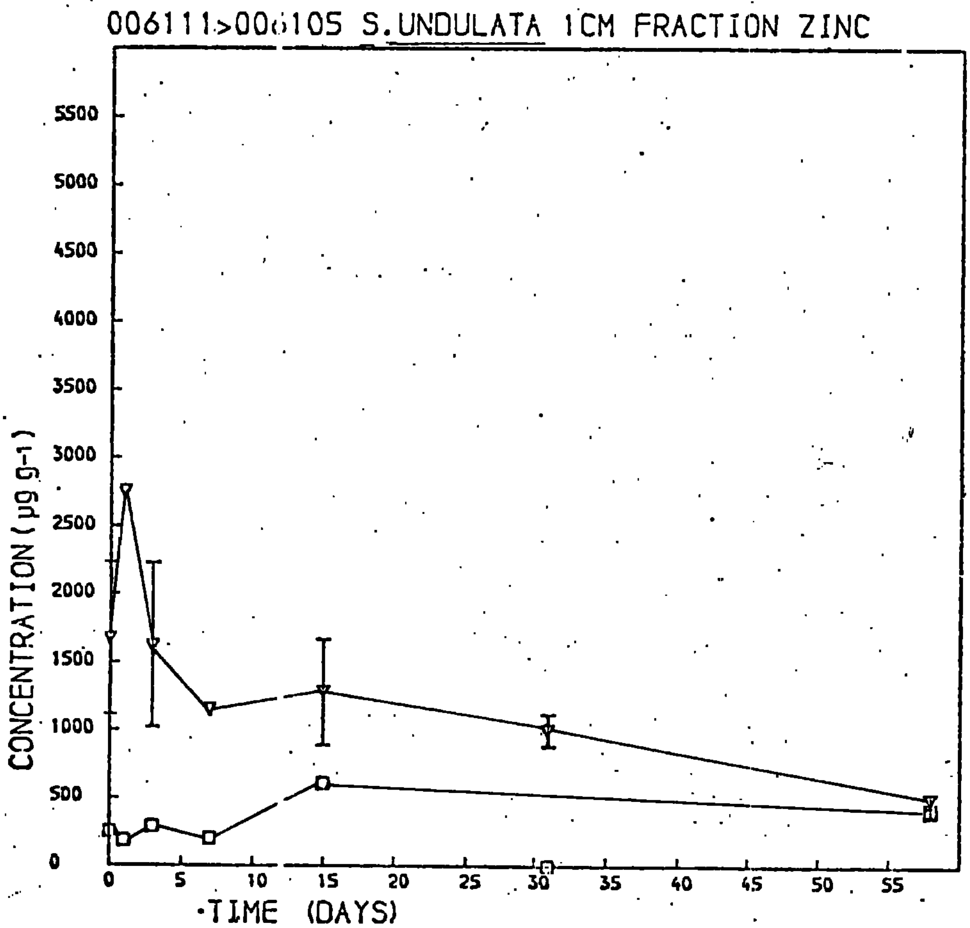
□ control material 1cm fraction

■ control material 1-2cm fraction

▽ transplant material 1cm fraction

▼ transplant material 1-2cm fraction

See Table 25 for data (Appendix C).


006111>006105 S.UNDULATA 1CM FRACTION ZINC
CONCENTRATION (µg g-1)
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
40
45
50
55
TIME (DAYS)

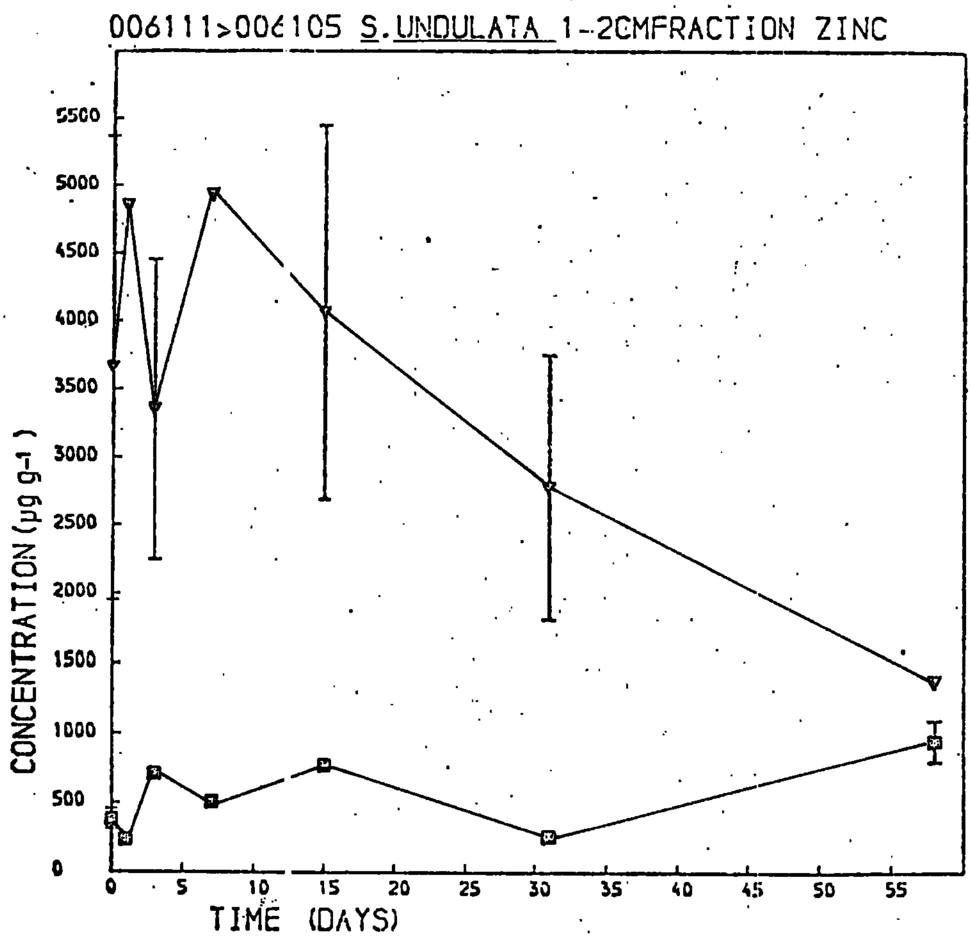

006111>006105 S.UNDULATA 1-2CMFRACTION ZINC
CONCENTRATION (µg g-1)
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
40
45
50
55
TIME (DAYS)

Figure 5. Concentration of lead in Scapania undulata ($\mu g\ g^{-1}$) as observed in reach 0061 05 during the long term transplant (22 May - 19 July).

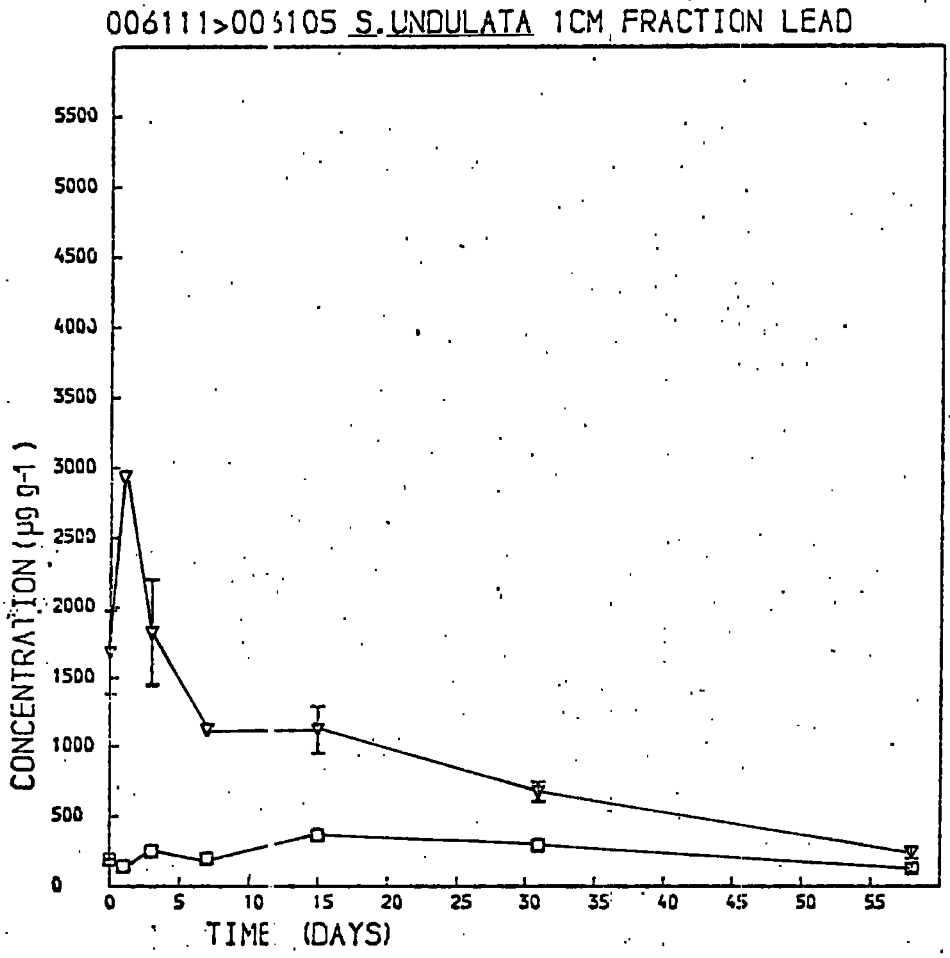
□ control material 1cm fraction

■ control material 1-2cm fraction

▽ transplant material 1cm fraction

▼ transplant material 1-2cm fraction

See Table 25 for data (Appendix C).


006111>00ƽ105 S.UNDULATA 1CM FRACTION LEAD
CONCENTRATION (µg g-1)
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0 5 10 15 20 25 30 35 40 45 50 55
TIME (DAYS)

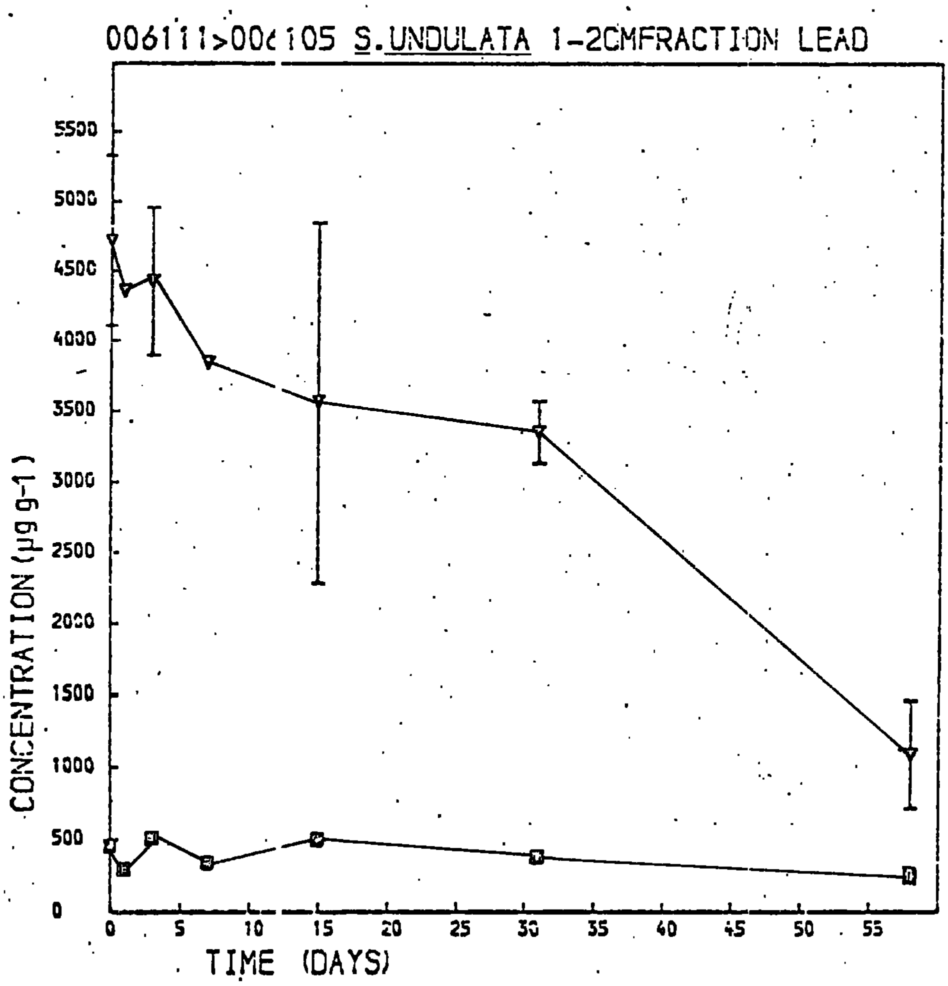

006111>006105 S.UNDULATA 1-2CMFRACTION LEAD
CONCENTRATION (µg g-1)
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0 5 10 15 20 25 30 35 40 45 50 55
TIME (DAYS)

Figure 6. Concentration of zinc in Scapania undulata ($\mu g\ g^{-1}$) as observed in reach 0061 11 during the long term transplant (22 May - 19 July).

□ control material 1cm fraction

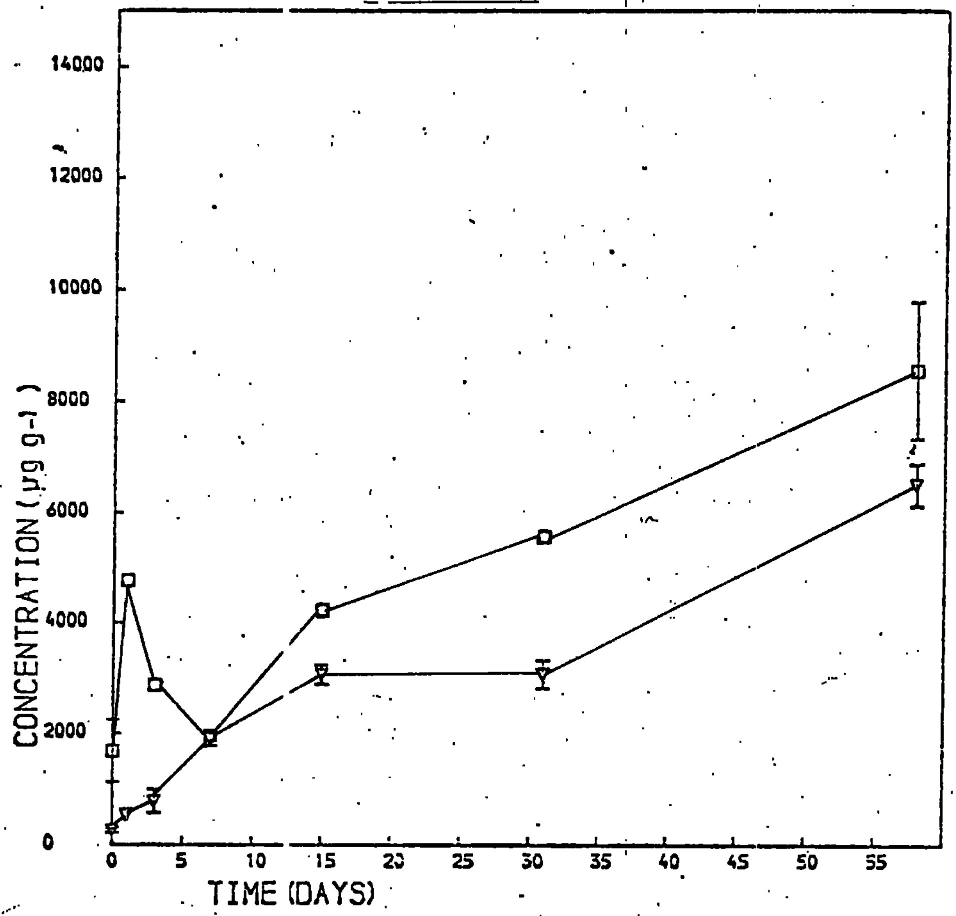
■ control material 1-2cm fraction

▽ transplant material 1cm fraction

▼ transplant material 1-2cm fraction

See Table 26 for data (Appendix C).

006105>006111 S.UNDULATA 1CM FRACTION ZINC



006105>006111 S.UNDULATA 1-2CMFRACTION ZINC

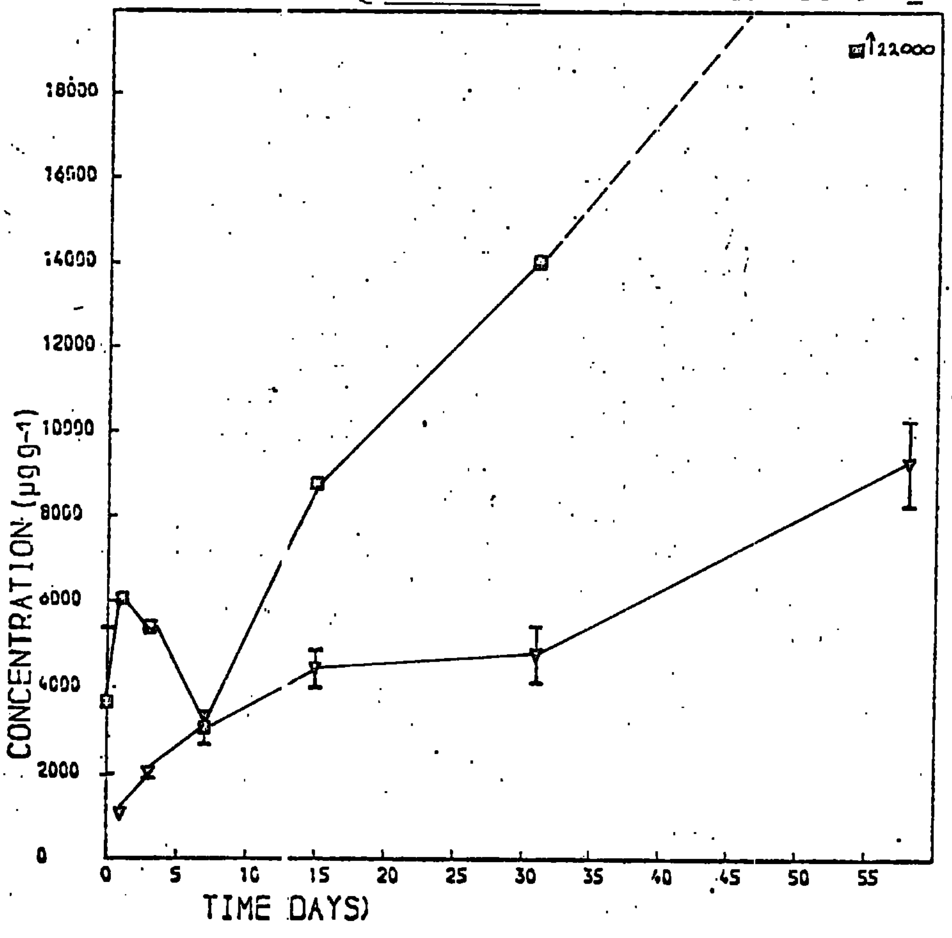


Figure 7. Concentration of lead in *Scapania undulata* ($\mu g\ g^{-1}$) as observed in reach 0061 11 during the long term transplant (22 May - 19 July).

□ control material 1cm fraction

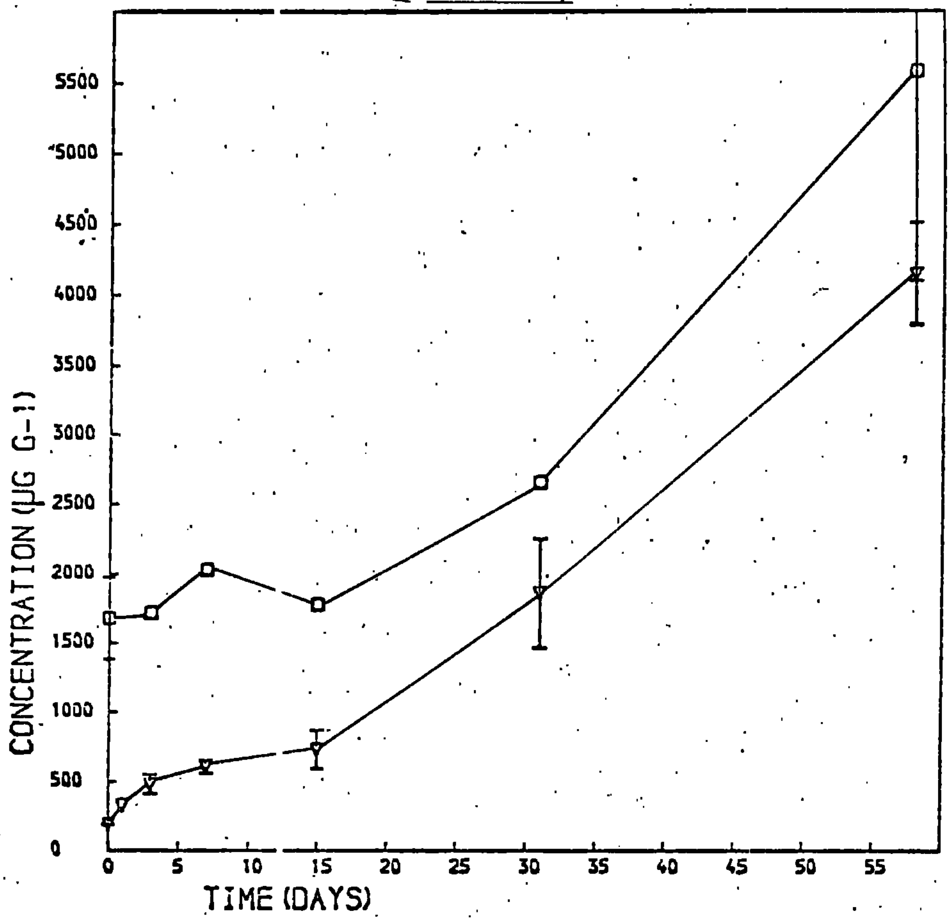
■ control material 1-2cm fraction

▽ transplant material 1cm fraction

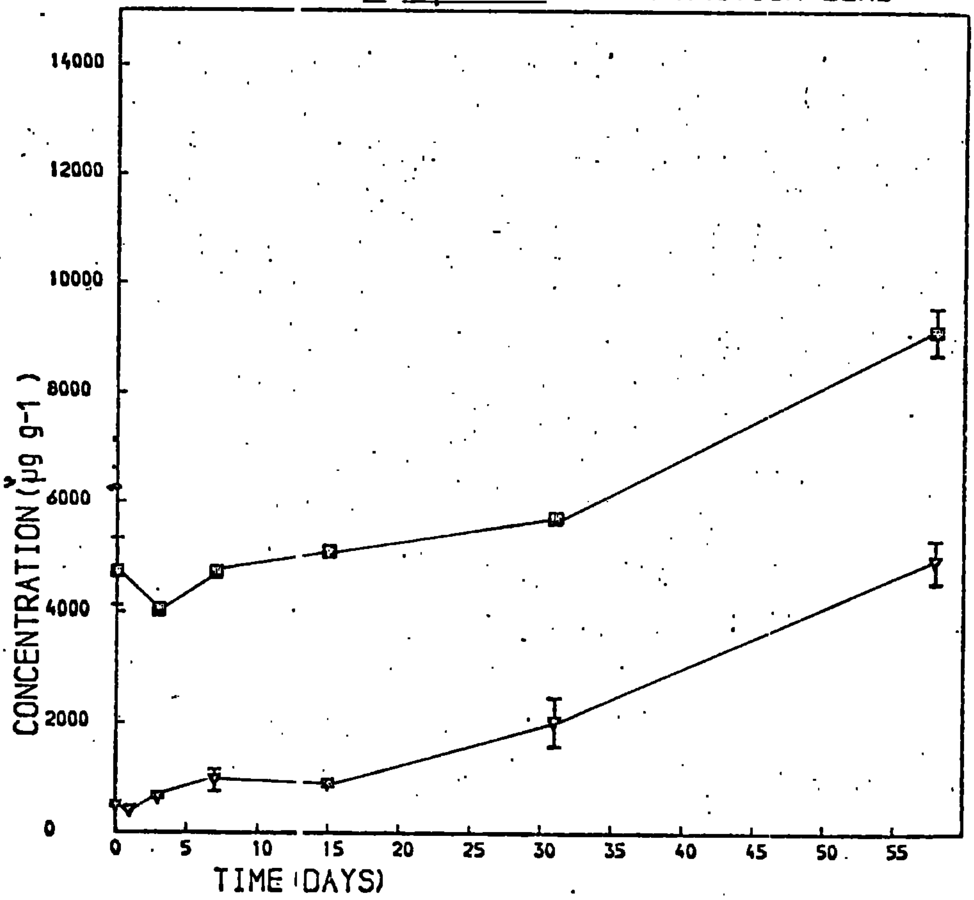
▼ transplant material 1-2cm fraction

See Table 26 for data (Appendix C).

006105 > 006111 S.UNDULATA 1CM FRACTION LEAD



006105>006111 S.UNDULATA 1-2CMFRACTION LEAD

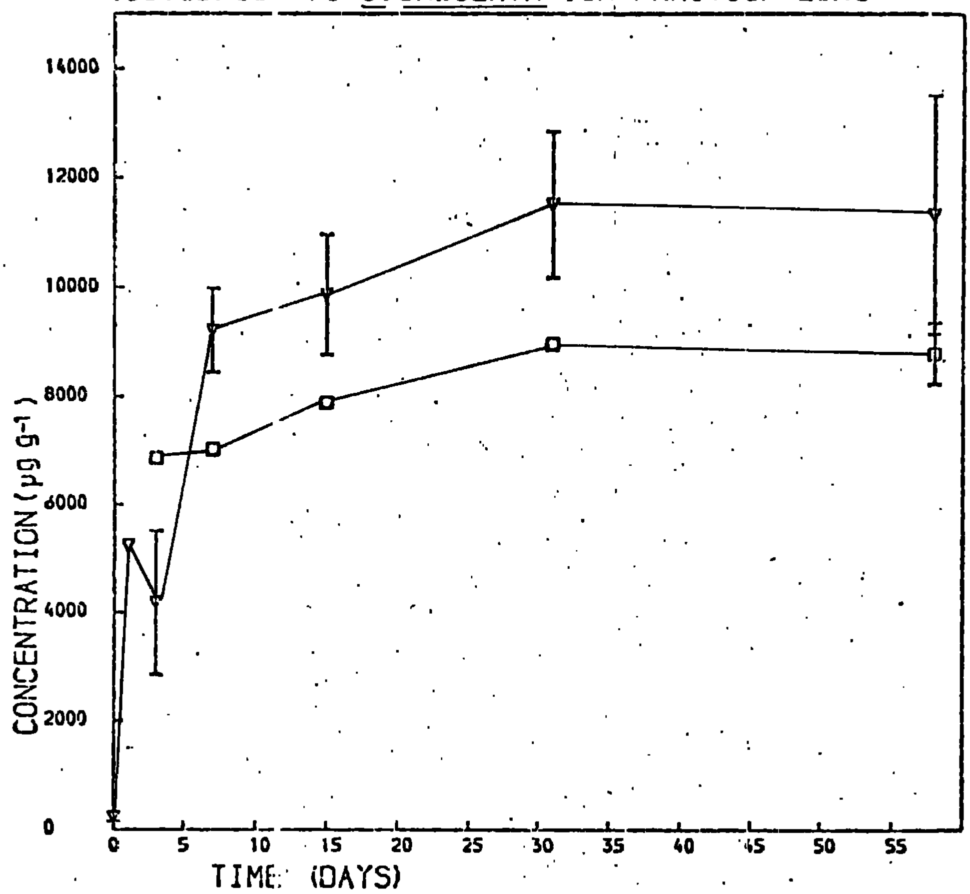


<u>Figure 8</u>. Concentration of zinc in <u>Scapania undulata</u> ($\mu g\ g^{-1}$) as observed in reach 0071 98 during the long term transplant (22 May - 19 July).

- □ control material 1cm fraction
- ■ control material 1-2cm fraction
- ▽ transplant material 1cm fraction
- ▼ transplant material 1-2cm fraction

See Table 27 for data (Appendix C).

006105>007198 S. UNDULATA 1CM FRACTION ZINC



006105>007198 S. UNDULATA 1-2CMFRACTION ZINC

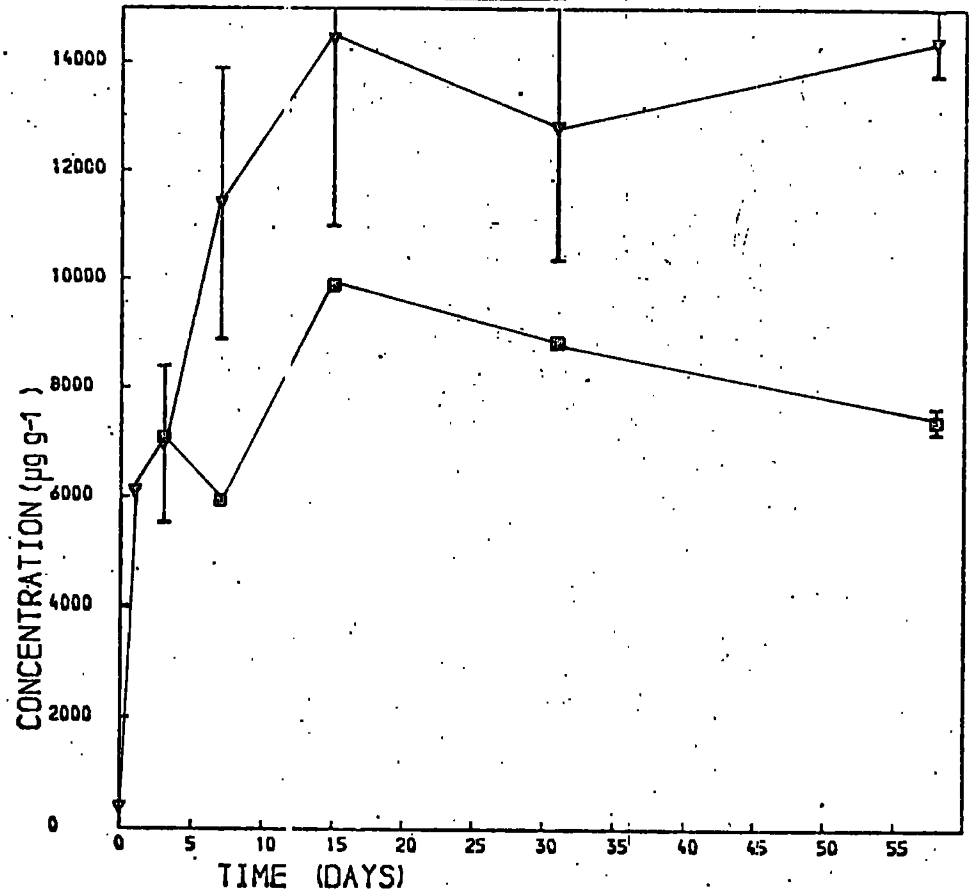


Figure 9. Concentration of lead in Scapania undulata (µg g^{-1}) as observed in reach 0071 98 during the long term transplant (22 May - 19 July).

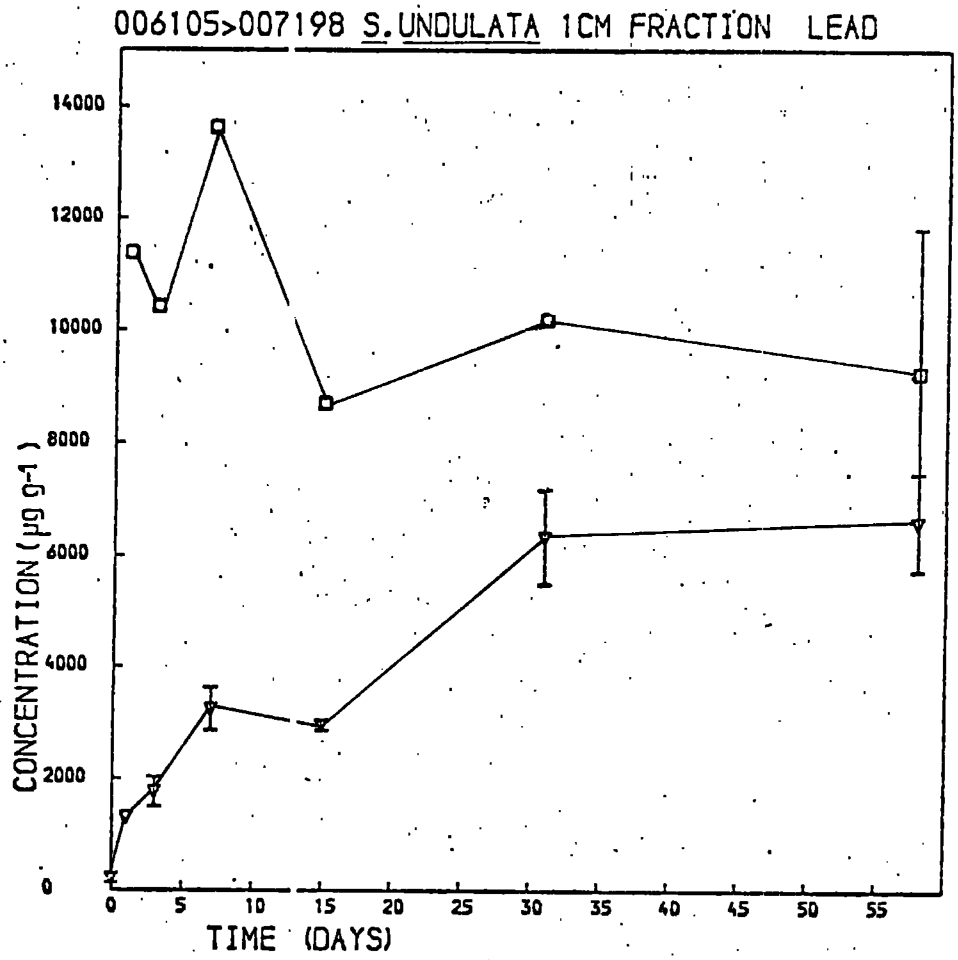
□ control material 1cm fraction

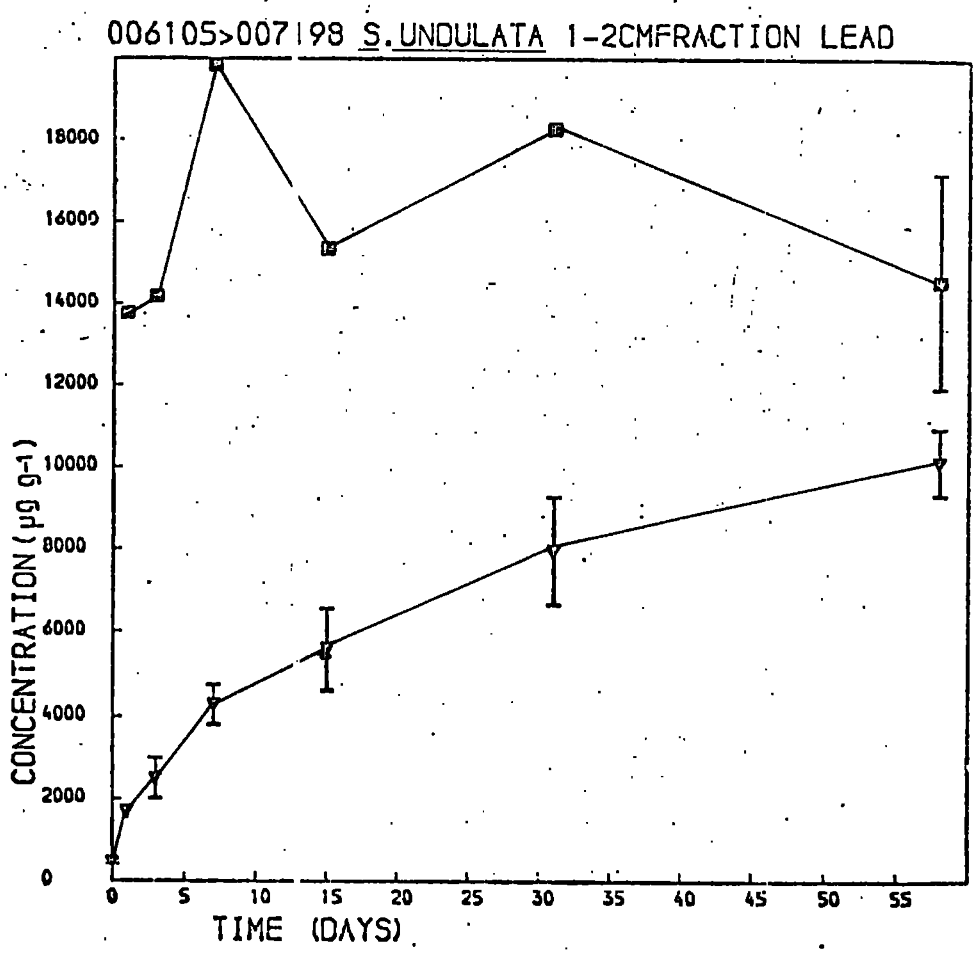
■ control material 1-2cm fraction

▽ transplant material 1cm fraction

▼ transplant material 1-2cm fraction

See Table 27 for data (Appendix C).


006105>007198 S.UNDULATA 1CM FRACTION LEAD
CONCENTRATION (µg g-1)
TIME (DAYS)
0
2000
4000
6000
8000
10000
12000
14000
0
5
10
15
20
25
30
35
40
45
50
55


006105>007198 S.UNDULATA 1-2CMFRACTION LEAD
CONCENTRATION (µg g-1)
TIME (DAYS)
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
0
5
10
15
20
25
30
35
40
45
50
55

<u>Figure 10.</u> Concentration of zinc and lead in *Chiloscyphus polyanthus* var. *rivularis* observed in reach 0061 05 during the long term transplant (22 May - 19 July).

◊ control material 1cm fraction

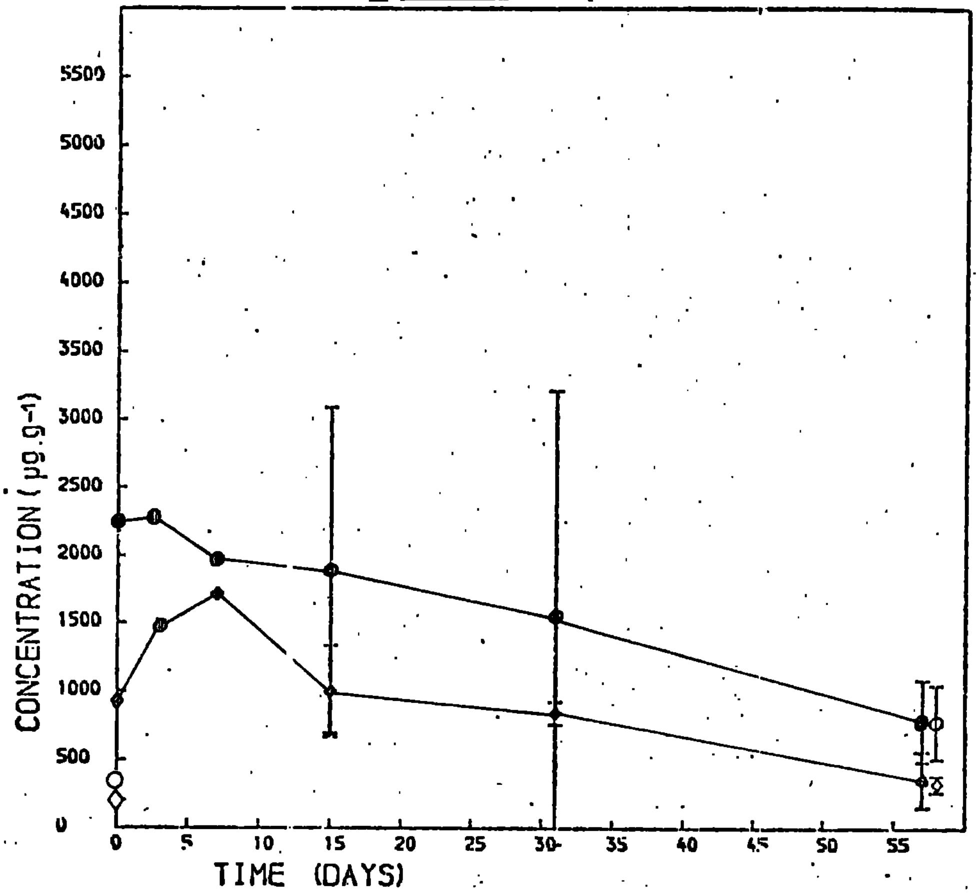
• control material 1-2cm fraction

♦ transplant material 1cm fraction

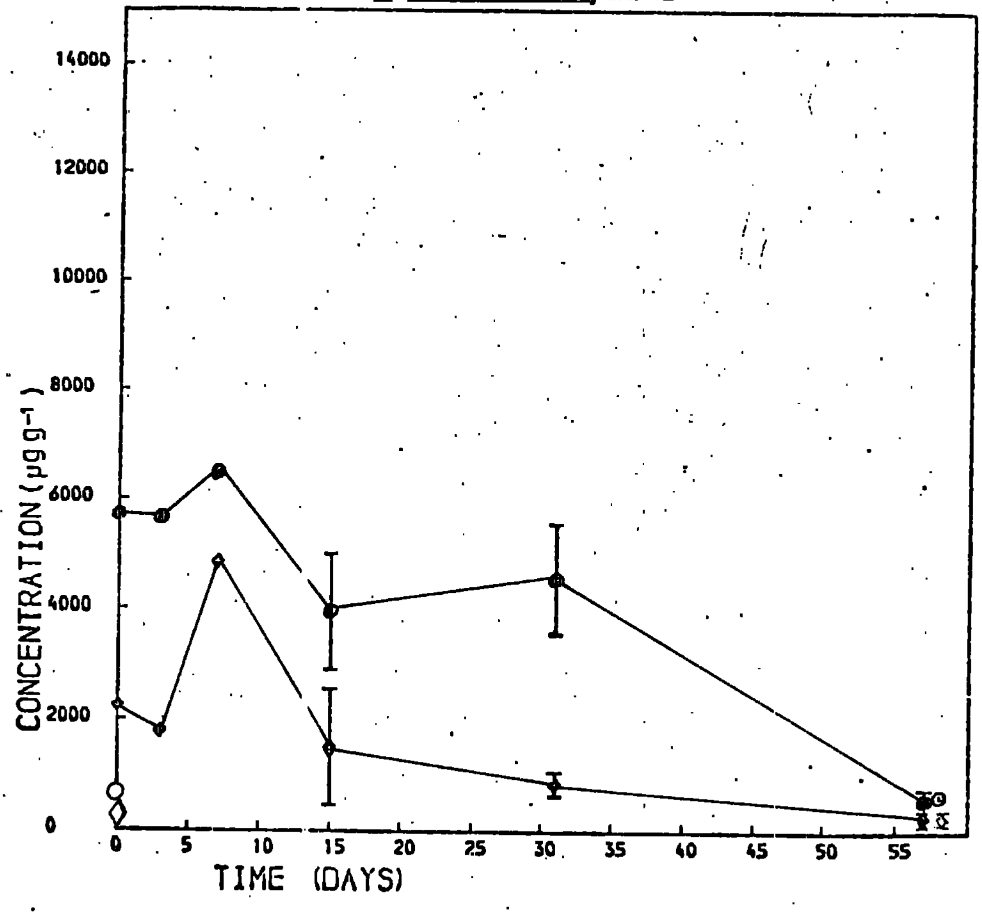
◉ transplant material 1-2cm fraction

See Table 28 for data (Appendix C).

006111>006105 C. POLYANTHUS, ZINC



006111>006105 C. POLYANTHUS, LEAD



<u>Figure 11</u>. Concentration of zinc and lead in *Chiloscyphus polyanthus* var. *rivularis* ($\mu g\ g^{-1}$) as observed in reach 0061 11 during the long term transplant (22 May - 19 July).

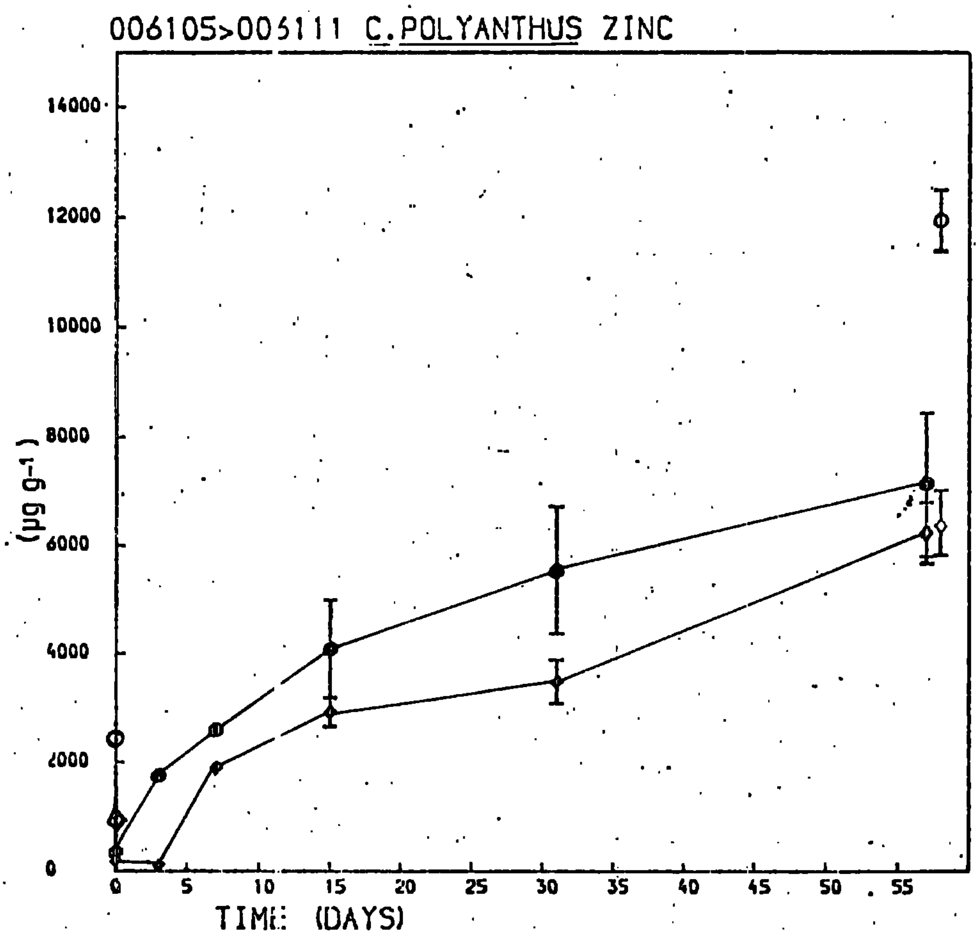
◊ control material 1cm fraction

o control material 1-2cm fraction

⧫ transplant material 1cm fraction

● transplant material 1-2cm fraction

See Table 29 for data (Appendix C).


006105>006111 C.POLYANTHUS ZINC
14000
12000
10000
8000
6000
4000
2000
0
(µg g⁻¹)
0
5
10
15
20
25
30
35
40
45
50
55
TIME (DAYS)

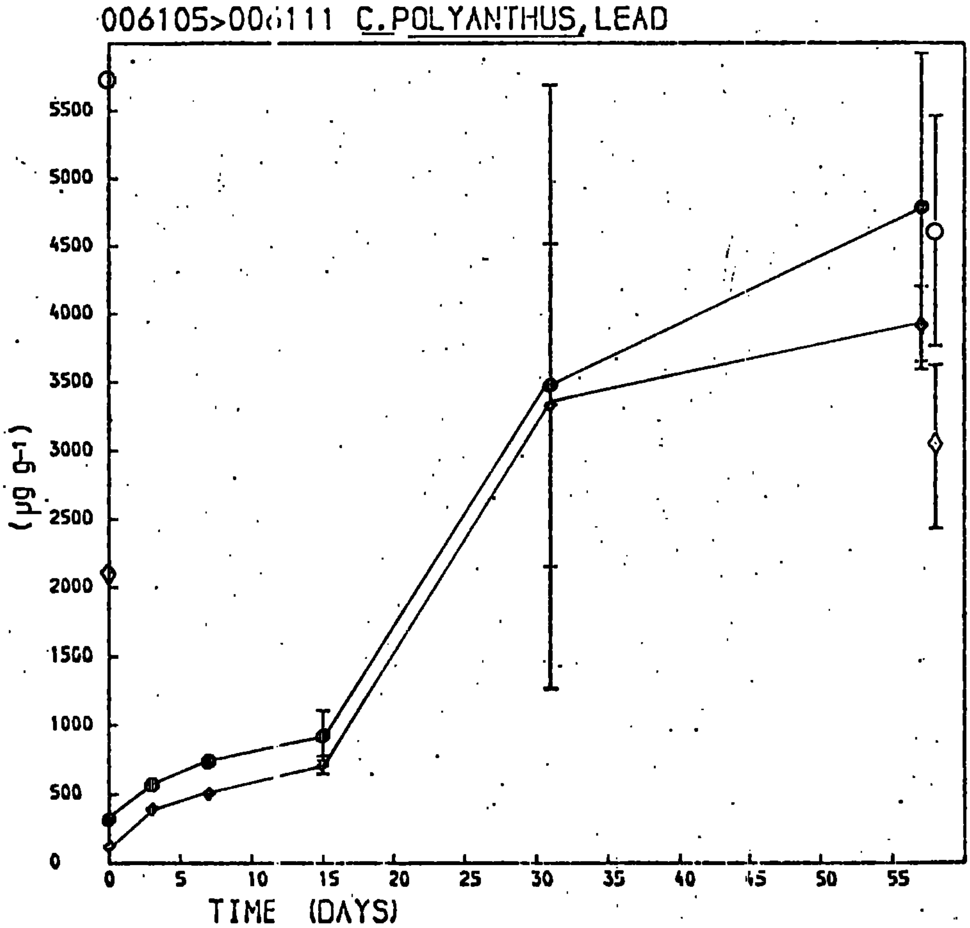

006105>006111 C.POLYANTHUS, LEAD
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
(µg g⁻¹)
0
5
10
15
20
25
30
35
40
45
50
55
TIME (DAYS)

Figure 12. Ratio diagrams: the level of percentage lead (Pb / (Pb + Zn).100) held by Scapania undulata during the long term transplant.

B reach 0061 05

A reach 0061 11

⬭ control material

⬬ transplant material

o filtered water sample

● total water sample

See Table 30 for data (Appendix D).

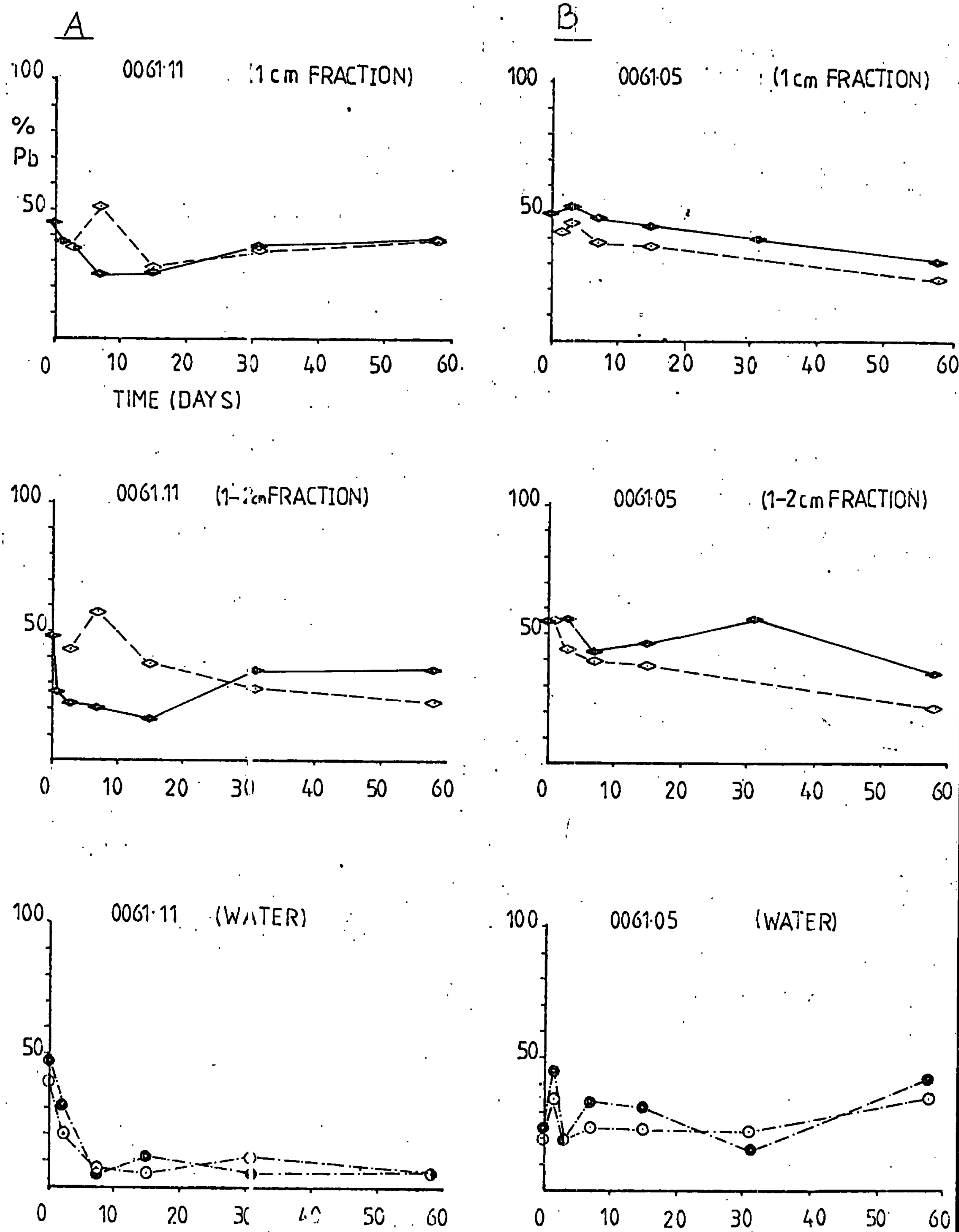
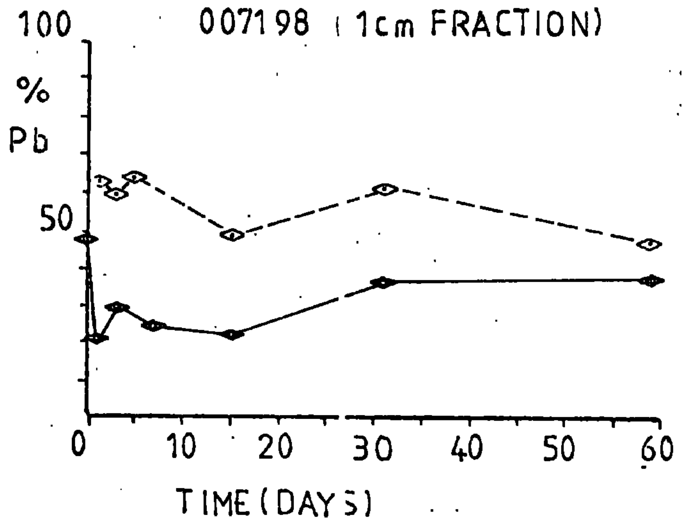
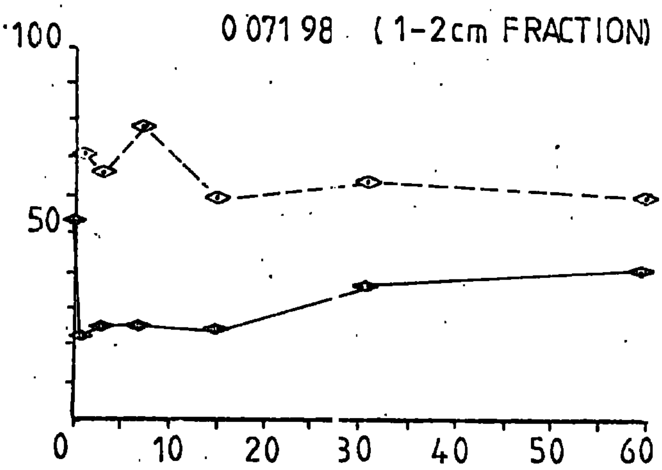

A
B
0061·11
(1 cm FRACTION)
% Pb
TIME (DAYS)
0061·05
(1 cm FRACTION)
0061.11
(1–2cm FRACTION)
0061·05
(1–2cm FRACTION)
0061·11
(WATER)
0061·05
(WATER)

Figure 13. Ratio diagrams: the level of percentage lead (Pb / (Pb + Zn).100) held by *Scapania undulata* in reach 0071 98 during the long term transplant.

- ◇ control material
- ◆ transplant material
- o filtered water sample
- ● total water sample

See Table 30 for data (Appendix D).


007198 (1cm FRACTION)
100
%
Pb
50
0
10
20
30
40
50
60
TIME (DAYS)


007198 (1-2cm FRACTION)
100
50
0
10
20
30
40
50
60

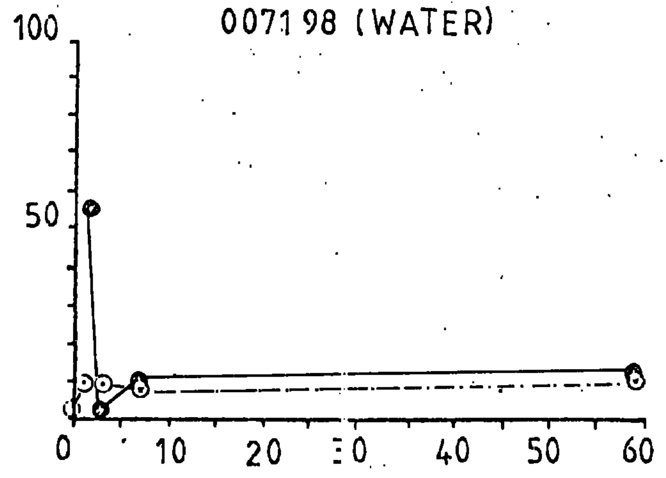

007198 (WATER)
100
50
0
10
20
30
40
50
60

Figure 14. Ratio diagrams: the level of percentage lead (Pb / (Pb + Zn).100) held by *Chiloscyphus polyanthus* var. *rivularis* during the long term transplant.

B reach 0061 05

A reach 0061 11

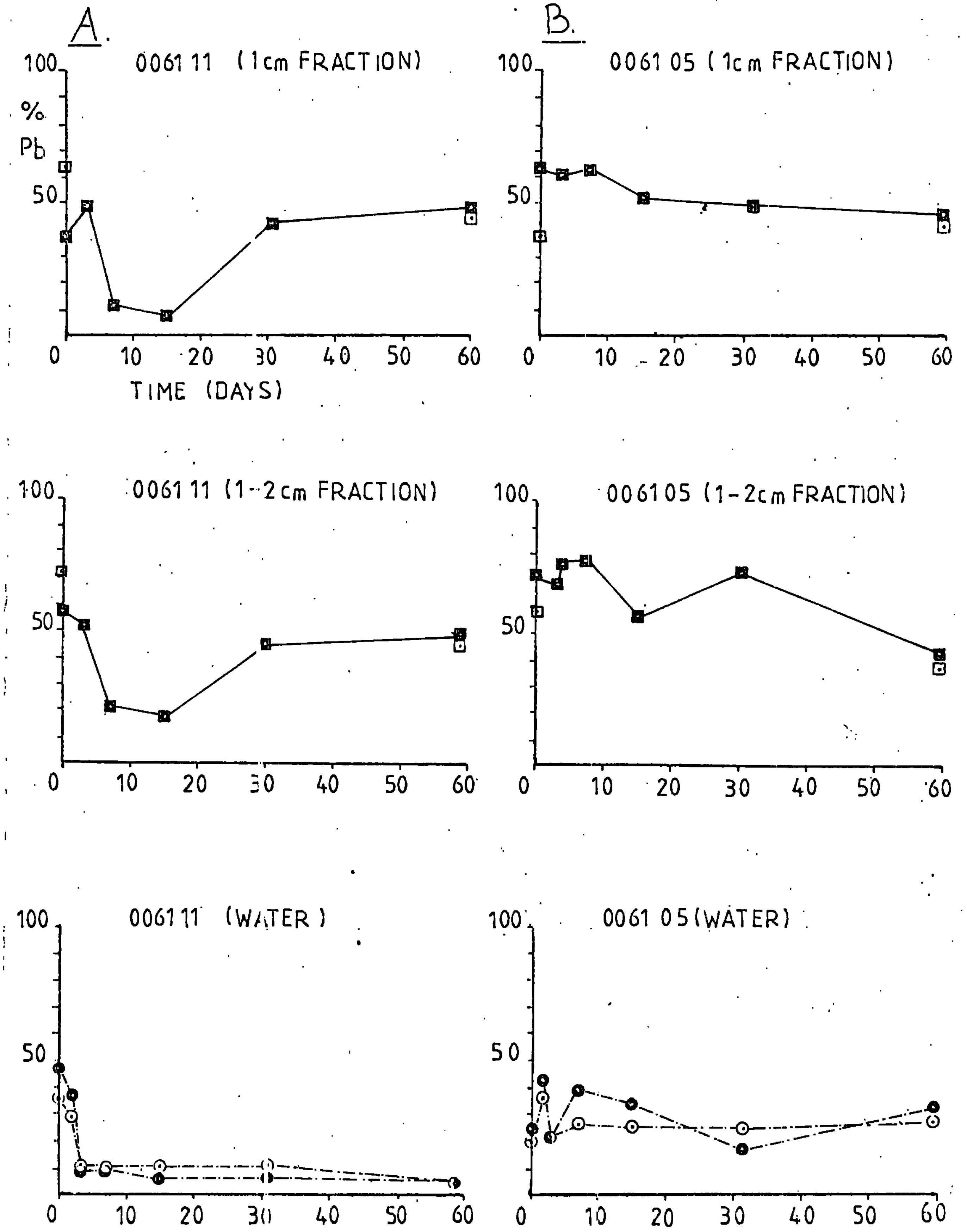
□ contol material

■ transplant material

○ filtered water sample

● total water sample

See table 30 for data (Appendix D).


A.
B.
0061 11 (1cm FRACTION)
0061 05 (1cm FRACTION)
%
Pb
100
50
0
10
20
30
40
50
60
TIME (DAYS)
0061 11 (1-2cm FRACTION)
006105 (1-2cm FRACTION)
0061 11 (WATER)
0061 05 (WATER)

<u>Figure 15</u>. Water temperature (°C) as observed over the short term transplant (2-4 July).

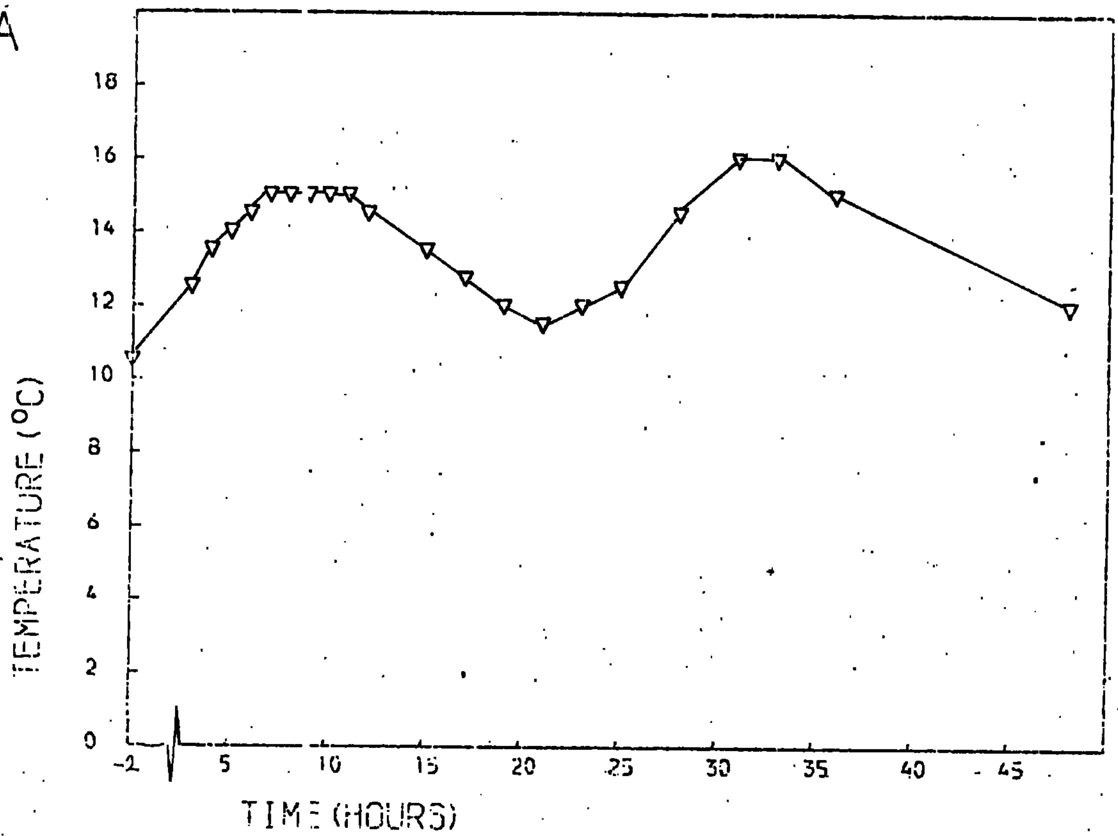
B reach 0061 05

A reach 0061 11

See Tables 35 and 36 for data (Appendix E).

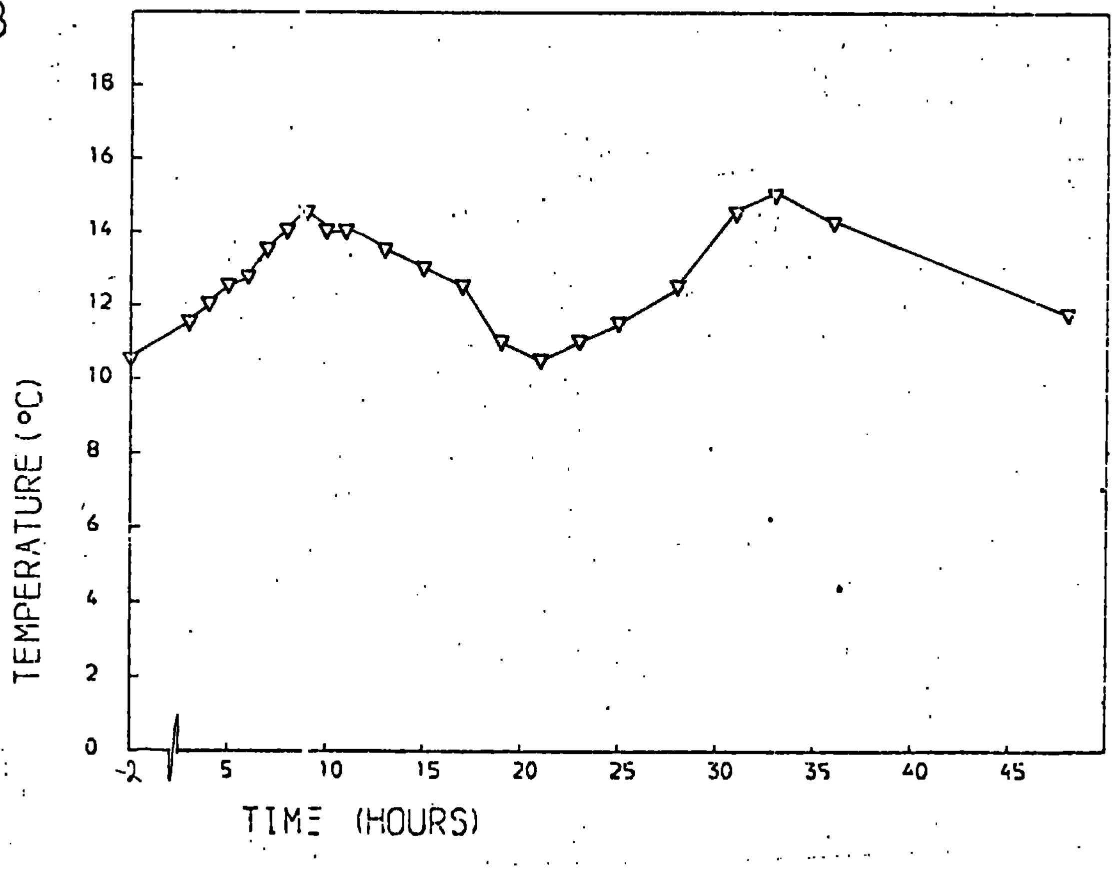
006111 TEMPERATURE PROFILE

A



TEMPERATURE PROFILE 006105 S.T. TRANSPLANT.

B

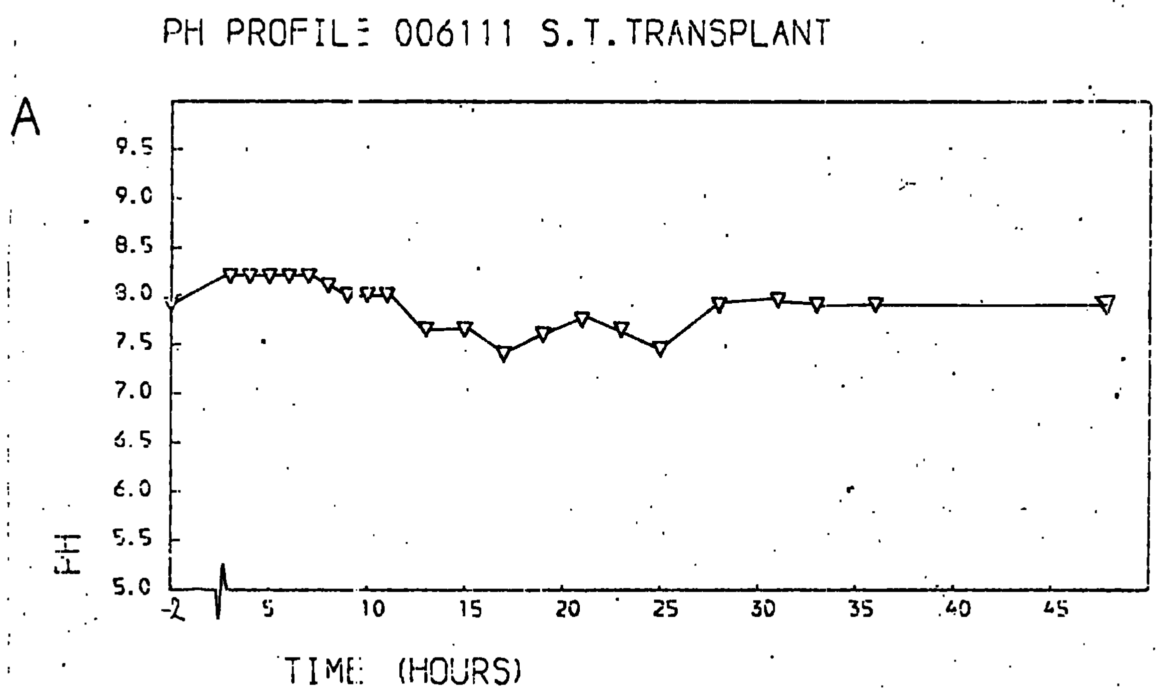


<u>Figure 16</u>. pH of water as observed over the short term transplant (2-4 July).

B reach 0061 05

A reach 0061 11

See Tables 35 and 36 for data (Appendix E).


PH PROFILE 006111 S.T.TRANSPLANT
A
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
PH
-2
5
10
15
20
25
30
35
40
45
TIME (HOURS)

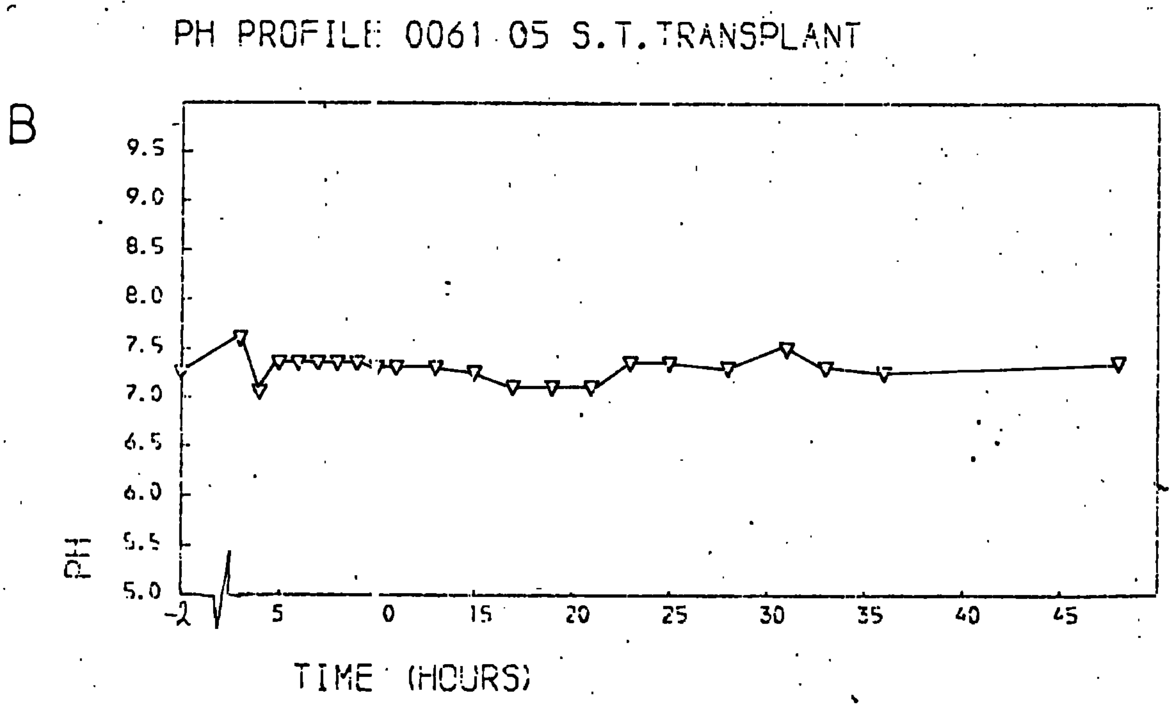

PH PROFILE 0061 05 S.T.TRANSPLANT
B
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
PH
-2
5
0
15
20
25
30
35
40
45
TIME (HOURS)

Figure 17. Concentration of lead in the water of reach 0061 05 (mg l^{-1}), expressed as 'total' concentrations, as observed over the short term transplant (2-4 July).

▽ 'total' water sample

See Table 35 for data (Appendix E).

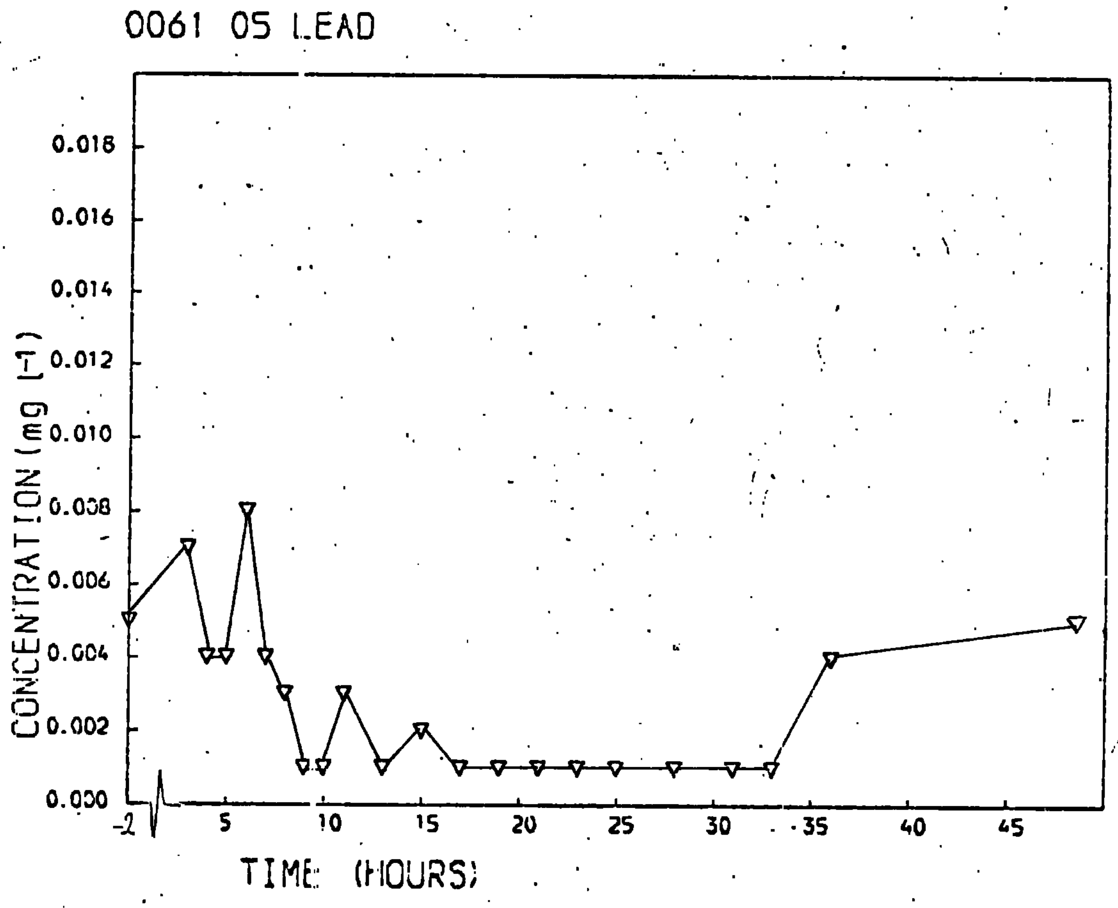
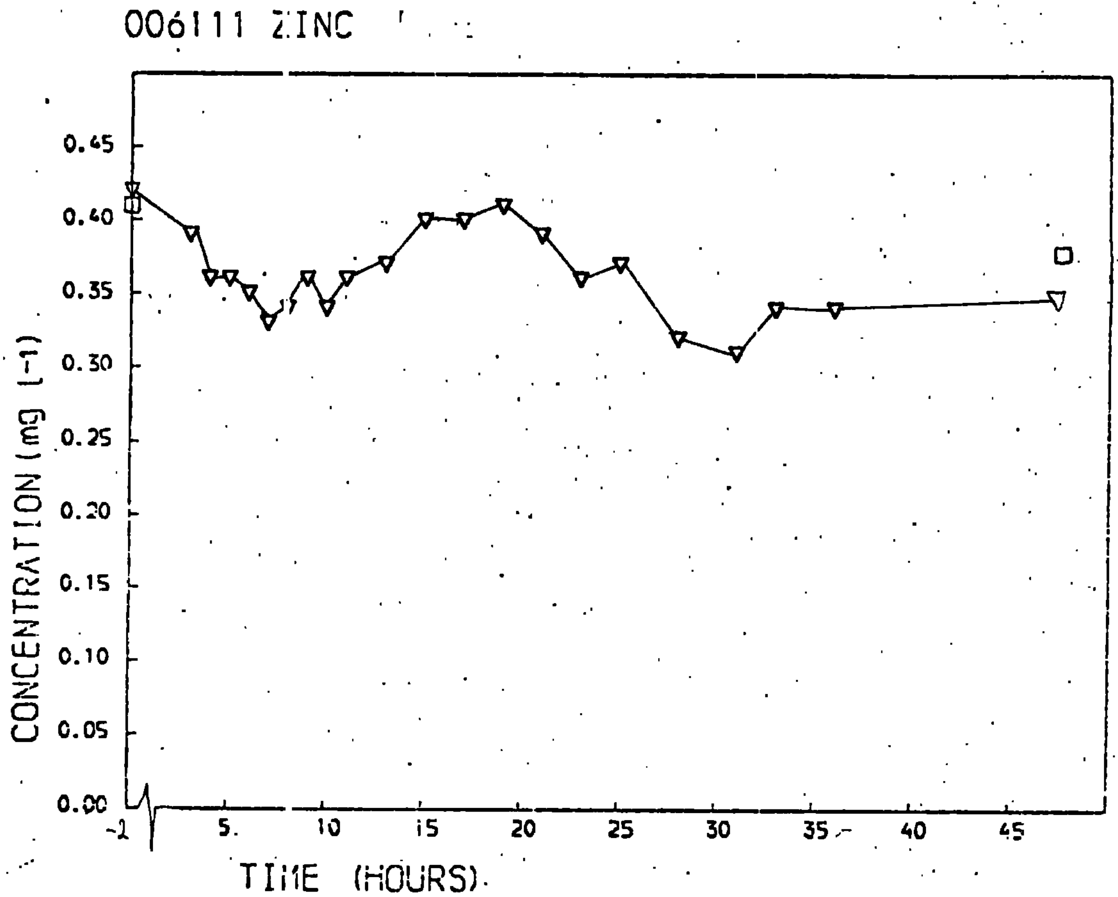

0061 05 LEAD
CONCENTRATION (mg l-1)
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
-2
5
10
15
20
25
30
35
40
45
TIME (HOURS)

Figure 18. Concentration of zinc and lead in the water of reach 0061 11 (mg l^{-1}), expressed as 'total' concentrations, as observed over the short term transplant (2-4 July).

□ 'filtered' water sample

▽ 'total' water sample

See Table36 for data (Appendix E).


006111 ZINC
CONCENTRATION (mg l-1)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-1
5.
10
15
20
25
30
35
40
45
TIME (HOURS)

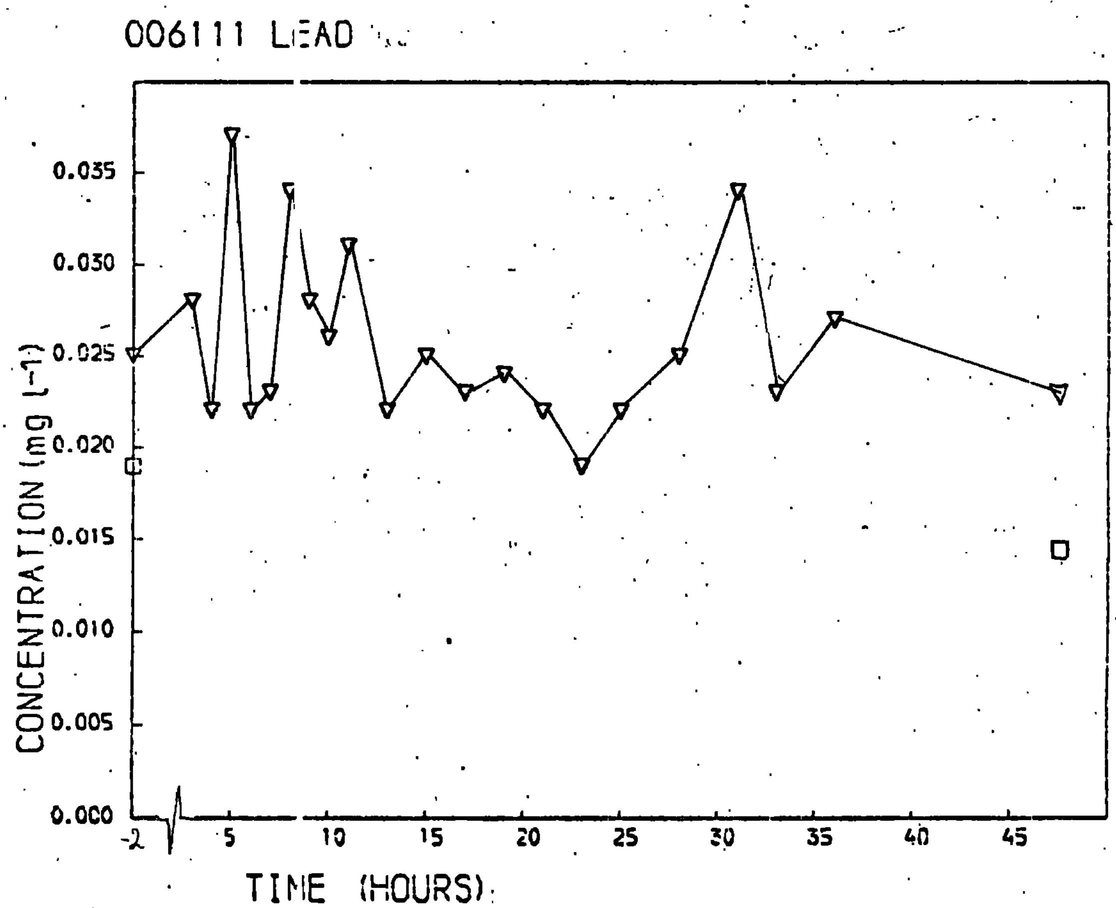

006111 LEAD
CONCENTRATION (mg l-1)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
-2
5
10
15
20
25
30
35
40
45
TIME (HOURS)

Figure 19. Concentration of zinc and lead in Scapania undulata (µg g^{-1}) as observed in reach 0061 05 during the short term transplant (2-4 July).

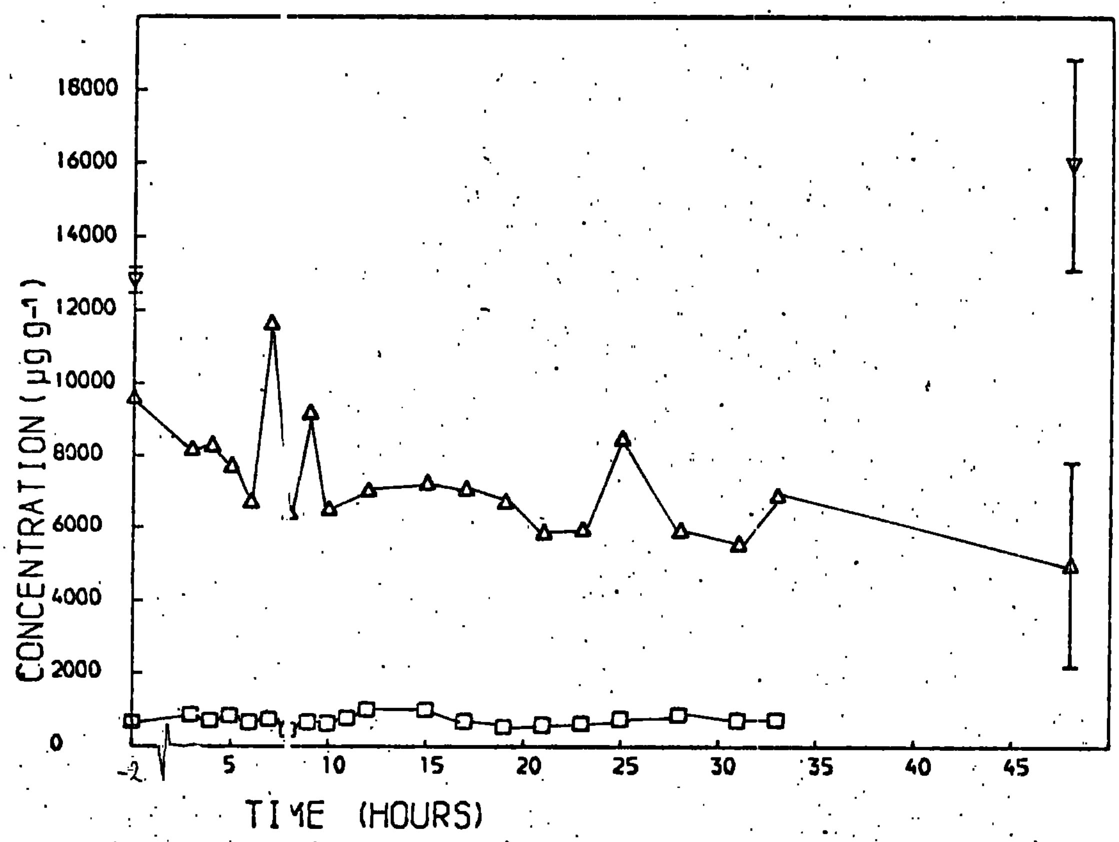
□ control material 1cm fraction

△ transplant material 1cm fraction

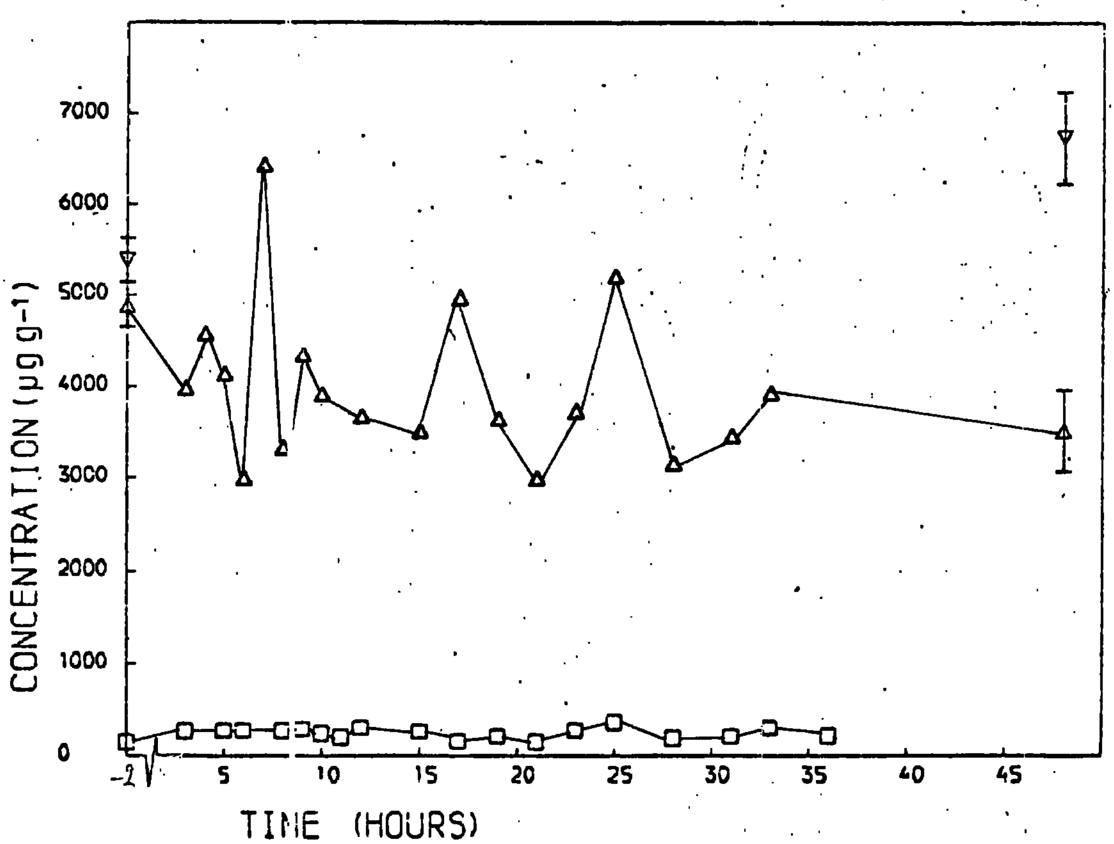
▽ transplant material 1-2cm fraction

See Table 37 for data (Appendix E).

006111>006105 S.T.TRANSPLANT ZINC



006111>006105 S.T.TRANSPLANT LEAD



<u>Figure 20</u>. Concentration of zinc and lead in <u>Scapania undulata</u> ($\mu g\ g^{-1}$) as observed in reach 0061 11 during the short term transplant (2-4 July).

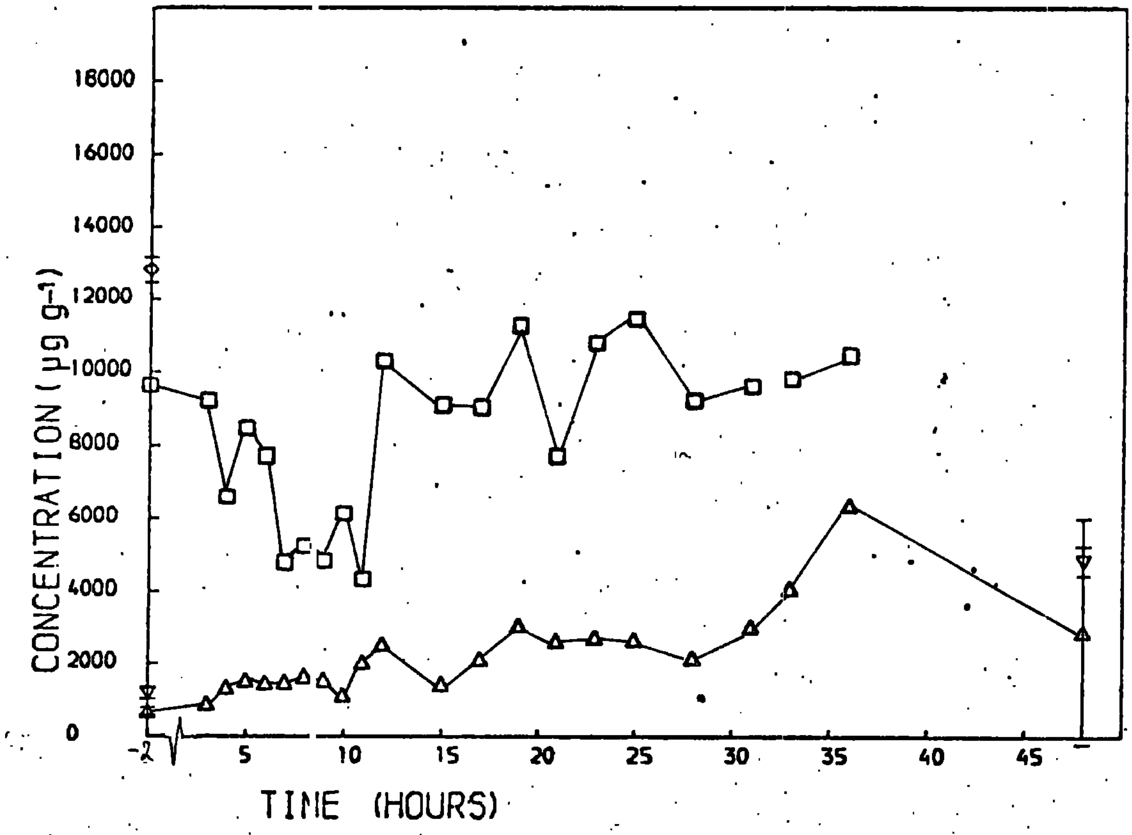
□ control material 1cm fraction

△ transplant material 1cm fraction

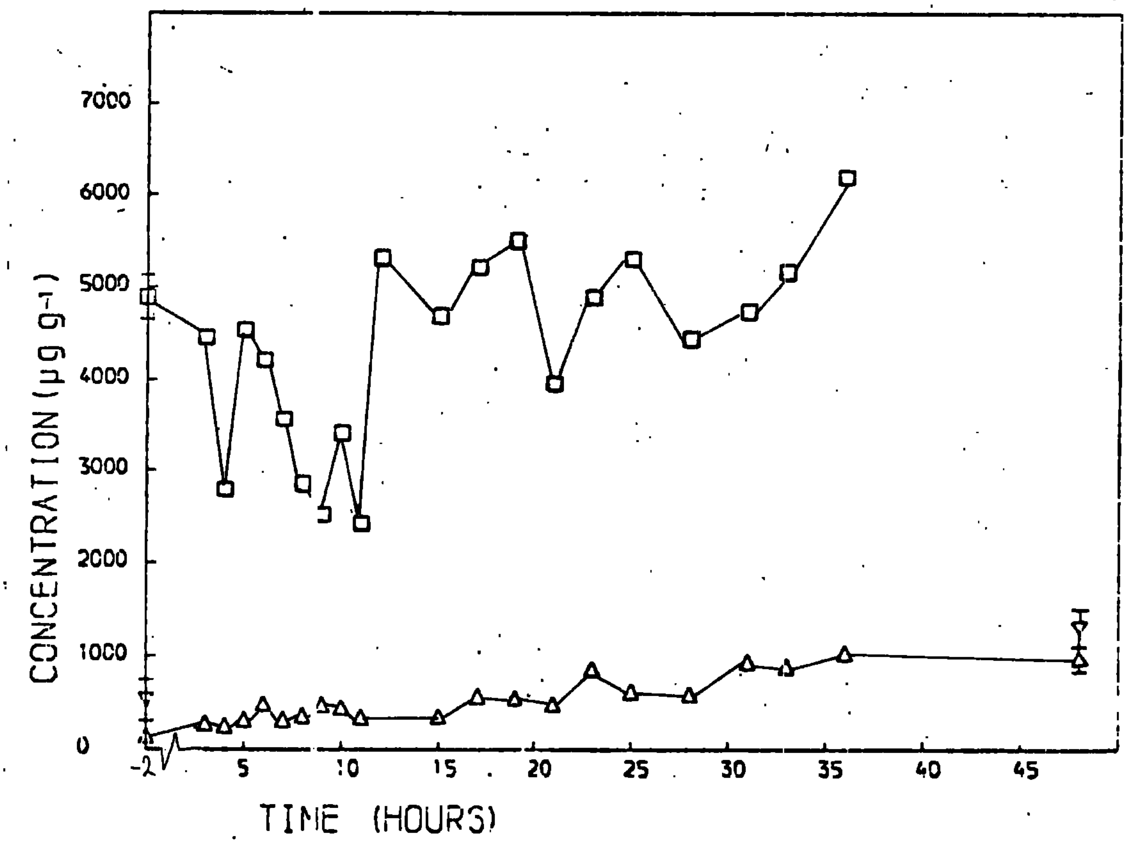
▽ transplant material 1-2cm fraction

See Table 38 for data (Appendix E).

006105>006111 S.T.TRANSPLANT ZINC



006105>006111 S.T.TRANSPLANT LEAD



CHAPTER 5

DISCUSSION

5.1 Enrichment ratios

An important aim of this report was to assess the stability of the relationship between the zinc and lead concentrations in the water and those accumulated by Scapania undulata. In Chapter 1 (Section 1.4) the use of enrichment ratios was introduced as a means of expressing this relationship in a quantitative manner. If the enrichment ratio remains constant over a wide range of ambient metal concentrations, then this greatly enhances the potential usefulness of a monitor.

Examples from the literature (Table 12) of enrichment ratios for Scapania range from 1145 to 11000, which corresponds to a range of aqueous zinc concentrations of 0.2 to 0.015 mg l^{-1}. The enrichment ratios recorded during the present study (Table 13) were in broad agreement with these figures. The values for each site varied over approximately one order of magnitude, a fact which is reflected by the relatively high coefficients of variance.

If Scapania is to be used as a monitor of ambient metal concentrations, the amount of variation in its enrichment ratios would pose difficulties. The interpretation of the zinc and lead concentrations of the monitor could not be undertaken with any degree of accuracy. If however, the causes of the variation could be determined, then it may be possible to compensate for their effects, or limit the monitor's use to those situations where these effects are minimal.

5.2 Zinc and lead in the controls

The concentrations of zinc and lead in the water of the River Derwent and Bolts Burn fell over the study period. The only exception to this was for reach 0061 11 where the zinc concentration increased two fold by Day 58 (Figures 2 and 3). The controls were expected to reflect any change in the ambient metal concentration. This did occur with

<u>Figure 20</u>. Concentration of zinc and lead in <u>Scapania</u> <u>undulata</u> ($\mu g\ g^{-1}$) as observed in reach 0061 11 during the short term transplant (2-4 July).

□ control material 1cm fraction

△ transplant material 1cm fraction

▽ transplant material 1-2cm fraction

See Table 38 for data (Appendix E).

Table 12. Concentrations of zinc quoted in the literature for Scapania undulata and surrounding water with the resultant enrichment ratio.

author	site	water Zn (mg l^{-1})	Scapania Zn ($\mu g\ g^{-1}$)	enrichment ratio
Dunker (1967)	M. Grain Sike	0.031 0.126	129 452	4161 3587
Lloyd (1967)	M. Grain Sike	0.131 0.015	150 266	1145 17733
Harding (1978)	River Derwent	0.021 0.272	150 2992	36714 11000

Table 13. Enrichment ratios for zinc and lead found in this study for Scapania undulata (calculated for controls).

Scapania fraction	site	water (range mg l^{-1})	enrichment ratios mean	S.D.*	C.V.*	range
lead						
1cm	0061 05	0.005–0.031	32600	15000	64%	4700– 58600
1–2cm			38000	26100	68%	9600– 75800
1cm	0061 11	0.019–0.055	68600	25500	37%	23300– 93600
1–2cm			217000	95900	44%	122500–381000
1cm	0071 98	0.037–0.01	179500	51700	28%	94900–235400
1–2cm			272200	108400	40%	114700–414900
zinc						
1cm	0061 05	0.016–0.06	8700	5900	68%	3100– 20300
1–2cm			23100	19200	83%	4000– 59500
1cm	0061 11	0.14 –0.58	15300	10400	68%	4600– 34000
1–2cm			32300	16100	50%	8200– 56200
1cm	0071 98	0.84 –1.98	6600	3400	52%	3500– 10500
1–2cm			7000	3200	45%	3004– 10200

S.D.* = Standard deviation   C.V.* = coefficient of varience

Scapania in the following cases: the lead concentrations of both fractions in reaches 0061 05 and 0071 98. The remaining controls did not, however, reflect the general fall in aqueous metal concentrations. Most controls (Scapania and Chiloscyphus) displayed a consistent rise in the level of accumulated zinc and lead (Tables 7-9; Figures 4-10).

The native populations of Scapania in reaches 006105 and 0061 11 were sampled during the preliminary survey on the 27 April (Table 3). The metal concentrations on the 27 April can, therefore, be compared with those at the start of the transplant experiment on the 22 May (Table 13).

Table 13. Zinc and lead in Scapania ($\mu g\ g^{-1}$) and river water ($mg\ l^{-1}$) on 27 April (preliminary survey) and 22 May (start of long term transplant).

	27 April		22 May	
	0061 05	0061 11	0061 05	0061 11
zinc				
water (filtered)	0.024	0.152	0.03	0.2
Scapania				
1cm fraction	255	590	235	1674
1-2cm fraction	446	766	383	3659
lead				
water (filtered)	0.005	0.024	0.01	0.023
Scapania				
1cm fraction	not	1218	not	1678
1-2cm fraction	available	2163	available	4721

Over the period from 27 April to the 22 May, the zinc and lead concentration in Scapania in reach 0061 11 increased. This could be a reflection of the aqueous zinc and lead concentrations over the intervening period; certainly the ambient zinc concentration was slightly higher on 22 May. This change in the accumulated metal concentration is, however, consistent with the increase in zinc and lead concentrations in the controls during the L.T. Transplant.

Four possible explanations may be put forward to explain the observed increase in the zinc and lead concentration of the controls.

i) It is a reflection of increasing zinc and lead concentrations in the water, or an increase in their availability due to some change in speciation. There is little evidence to support this from the water analyses, but as only spot samples were taken there is room for doubt. Grimshaw, Lewin and Fuge (1976) in a study of dissolved zinc in the River Ystyth, warn against infrequent sampling because of the potential variability shown by zinc levels.

ii) The bryophytes show a continuous net rate of metal accumulation. As a consequence, the level of zinc and lead would be proportional to the length of time over which the bryophyte was exposed . Older bryophyte material would, therefore, have higher zinc and lead concentrations, while the material transplanted to reaches of higher metal contamination might be unable to catch up with the levels attained by the native populations.

iii) A decrease in the rate of growth over the period could, by reducing the rate of incorporation of new material, result in an apparent increase in accumulated metal levels. Over the period of study the abundance and luxuriance of _Scapania_ decreased (Section4.2). If the growth rate followed this pattern, it should be detectable in the growth experiment (Table 14). The rate of growth in the second stage of the growth experiment was slower than that in the first stage ; the difference was however small.

Table 14. Mean rate of growth (mm d^{-1}) of Scapania undulata over the two stages of the growth experiment. Calculated from the control samples for all reaches (Table 41 and 40).

	days after start of experiment	
	0 - 24	25 - 51
mean (mm d^{-1})	0.055	0.048
standard deviation	$\pm$ 0.049	$\pm$ 0.038

iv) A metabolic/morphological change occurs in the liverworts, resulting in an increased rate of uptake. It is possible to calculate the rate of accumulation over the first three days of the L.T. Transplant and compare it to the rate over the S.T. Transplant (Tables 7 and 15). The S.T. Transplant was started 40 days after the beginning of the L.T. Transplant. The rate of accumulation would appear to have increased over this period.

Four possible theories were put forward to explain the increase in zinc and lead concentrations in the controls during the L.T. Transplant: of these the most plausible are (ii) and (iv). The possibility that Scapania exhibits a continuous net rate of accumulation would explain the behaviour of the controls and the fact that the transplants in reach 0061 11 did not attain the same metal concentration as the controls. It would also explain why the older 1-2cm fractions have a higher metal content than the younger 1cm fractions. The alternative explanation, that there is a change in the accumulation rate of zinc and lead, is supported by some of the transplant data. The transplant on the 22 May (start of L.T. Transplant) from reach 0061 05 to reach 0061 11, would have been expected to display a similar rate of accumulation to that found on the subsequent repeat of the transplant on the 2 July (S.T. Transplant). The increase in the accumulation rate revealed (Tables 7 and 15) can not be attributable to a change in the metal concentration of the water in reach 0061 11 as this full. The difference between the metal concentrations of the two reaches at the time of transplant increased, and this may be of importance(Table 16). Theory (iv) explains the behaviour of the controls and that of the transplants in reach 0061 11. It does not directly explain the differences between fractions.

It has been demonstrated that, over a certain range of ambient zinc (0.016 –0.58 mg l^{-1}) and lead concentrations (0.019–0.055 mg l^{-1}), the relationship between the accumulated metal level and the ambient level was subject to change over the period studied. The use of Scapania as a monitor of zinc and lead should be treated with caution until this phenomenon is investigated further.

5.3 Response time

The time taken for a monitor to reflect a change in the ambient metal concentration is an important characteristic determining the stability of its enrichment ratio. While the accumulated metal concentration is'out of phase' with the ambient metal concentration, the enrichment ratio will be distorted. The longer the response time of a monitor, the less accurate it will be in recording change.

The response time, to the changes in metal concentration resulting from transplantation, maybe defined as the time taken by the transplants to attain the same metal concentration as the controls. This statement assumes that the controls maintain a constant enrichment ratio. The assessment of the response rate of Scapania is hindered, therefore, by the increase in the concentrations of zinc and lead observed in some of the controls (Section 5.2). It was only possible, to make a rough estimate of the response rate of Scapania where the enrichment ratio was relatively stable.

Scapania transplanted into reach 0061 11 did not attain the same concentration of zinc and lead as the controls within 58 days. This was probably a consequence of the increase in the zinc and lead concentrations in the controls of reach 0061 11 (Figures 4 and 5; Table 5). In reach 0071 98, where the metal concentration of the controls was relatively stable, the concentration of zinc in the transplants increased beyond that of the controls by Day 7. The lead concentration of these transplants took 58 days to reach a similar concentration to that of the controls (1cm fraction). In reach 0061 05, where there was a small increase in the metal concentration of the controls, the transplants achieved a similar concentration by Day 58 (1cm fraction). Attainment of a similar concentration was, for the purpose of this discussion, defined as the point where the standard deviations of the means of concentrations overlapped. Using this definition, none of the 1-2cm fractions achieved a similar concentration to the controls, though the differences were small by Day 58.

The response time can only be estimated approximately for Chiloscyphus, as controls were taken only at the beginning and end of the transplant experiment. By Day 58, all but one of the transplants had concentrations very similar to those of the controls; the exception was the 1cm fraction of the material transplanted into reach 0061 11, where the zinc concentration of the controls had increased significantly (Figure 10).

In conclusion, Scapania did not respond rapidly to changes in the ambient zinc and lead concentration. Most of the transplants took at least 58 days to attain the same concentration as the controls. The effect of the increase in zinc and lead concentrations of the controls considerably complicated the situation. As a consequence the response time following a rise in ambient metal levels may have been overestimated.

5.4 Observed rates of accumulation and loss

The response time of a monitor is determined by: the magnitude of the change in ambient metal concentration and how the rate of this change compares to the rate of accumulation or loss of metals by the monitor. The rates of accumulation and loss of different metals are, therefore, an important characteristic of a monitor. If determined, it could be possible to understand and even predict the type of response which would result from changes in the ambient metal concentrations.

The observed rates of accumulation and loss, which result from transplantation, are probably a consequence of at least three factors.

i) The difference in ambient metal concentrations between the 'recipient' and 'donor' transplant sites at the time of transplantation.

ii) The concentration of the metals in the 'recipient' site over the period of study.

iii) The intrinsic rates of accumulation and loss characteristic of a particular plant.

The effect of the first two factors must be accounted for, so that an assessment of the intrinsic rates characteristic of Scapania can be made.

5.41 Rates of accumulation and loss

The rate of accumulation of zinc and lead appeared, during the L.T. Transplant, to be greater than their rate of loss. The observed rate of change in the metal content of Scapania was greater when the transplant involved an increase in the aqueous metal concentration (Figures 4 to 9). This can be clearly seen if the initial rates over the first three days are compared (Table 7).

The S.T. Transplant was undertaken to examine, in more detail, the changes over the first two days following transplantation(Section 3.51). The results indicated that the rate of loss was greater than the rate of accumulation of zinc and lead (Table 7), a reversal of the situation found in the L.T.Transplant. It is difficult to explain this discrepancy .

The rates of loss and accumulation were calculated from the reciprocal transplants between reaches 0061 05 and 0061 11. The rate of accumulation was determined from the transplant from 0061 05 to 0061 11, while the rate of loss was determined during the transplant fron reach 0061 11 to 0061 05. The change in ambient metal concentrations, to which these two transplants were subjected, was therefore identical in magnitude, but opposite in direction (one was subject to an increase in ambient metal concentrations and one to a decrease). The same transplants when undertaken 40 days later would have been expected to display the same relative rates. The absolute rates could well be different as the concentration of zinc and lead in the two reaches had changed (Tables 23,24. 35 and 36).

A possible explanation for the discrepancy between the results of the L.T. Transplant and the S.T. Transplant may be sought by examining the ambient metal levels subsequent to transplantation. If the assumption is made that the rate of accumulation or loss of zinc and lead is affected by the concentrations of these metals in the water, then any change in their concentration at the'recipient' transplant site would be of importance (Section 5.4 (ii)). If such a change occurred during one of the transplant periods, then this would affect the relative rates of accumulation and loss, and therefore possibly explain the differences between the short and long term transplants.

During the first three days of the L.T. Transplant there was a change in the aqueous zinc and lead levels, presumably as a consequence of the river being in spate during Day 1. Metal concentrations approximately doubled, except in reach 0061 11 where the concentration of zinc fell (Tables 23 and 24). The aqueous zinc and lead concentrations displayed an approximately diel pattern of variation during the S.T. Transplant (Tables

35 and 36). This presumably also occurred during the L.T. Transplant; comparison of the concentration every 24 hours shows that the concentration of zinc and lead remained stable during the S.T. Transplant.

Changes did occur in the zinc and lead concentration of the water following transplantation and these differed in the case of the long and short transplants. It is, however, difficult to accept that they were of sufficient magnitude to account for the difference in the relative rates of accumulation and loss observed between the two transplant experiments.

Within one day of being transplanted into reach 0061 05 the zinc and lead concentrations of Scapania had increased by 65% and 74% respectively (Figures 4 and 5). It seem possible, as reach 0061 05 has low ambient metal levels . that the values for Day 0 are inaccurate. If the rates of accumulation and loss ,for the initial period of the L.T. Transplant, are calculated from Day1 to Day 3 instead of from Day 0 to Day 3, then they are compatible with the rates displayed during the S.T. Transplant (Table 15).

A tentative conclusion is possible concerning the relative rates of accumulation and loss of zinc and lead, on the basis that, for the L.T. Transplant, the values for Day 0 are erroneous. The calculation of rates from Day 1 to Day 3 reveals that, over the initial period of the L.T. Transplant, the rate of loss is greater than the rate of accumulation. This is in agreement with what was found during the S,T. Transplant. Whether this applies over the full 58 Days can not be determined on the basis of the results.

5.42 Accumulation and loss of zinc and lead

Zinc appears to be accumulated and lost by Scapania at a greater rate than lead. This can be clearly seen in the initial period of the L.T. Transplant and over the S.T. Transplant (Tables 7 and 15). Over the full 58 days of the L.T. Transplant the apparent rate of zinc accumulation was greater than that of lead (Figures 6 and 9).

Table 15. Comparison of the rates of accumulation and loss of zinc and lead, by the 1cm fraction of Scapania, during the initial period of the L.T. Transplant and the S.T. Transplant. Figures calculated from the change in metal concentrations, over the period from Day 1 to Day 3 (L.T. Transplant) and over the two days of the S.T. Transplant.

	0061 11 - 0061 05		0061 05 - 0061 11	
	zinc	lead	zinc	lead
L.T. Transplant rate in $\mu g\ g^{-1}\ d^{-1}$	- 567	- 555	+ 123	+ 75
S.T. Transplant rate in $\mu g\ g^{-1}\ d^{-1}$	- 2300	- 700	+ 1110	+ 440

The change in ambient metal concentrations, to which the bryophytes were subjected on transplant, will influence the rate of accumulation or loss (Section 5.4 (i)). The change in zinc concentrations between reaches was substantially greater than the change in lead concentrations (Table 16). The greater rate of zinc accumulation and loss may, therefore, be a reflection of this.

A change in the ambient metal concentration of the 'recipient' site, subsequent to transplantation, will affect the rate of accumulation or loss of that metal by the monitor. If the changes in zinc and lead concentration in the water (Figures 2 and 3) are compared to the behaviour of the transplants (Figures 4 to 11), there would appear to be no obvious correlations. On the basis of the information available, it would seem that any changes in ambient metal concentrations were either too small or of too short a duration to have any substantial effect on the rates of accumulation or loss.

Table 16. The difference (at the time of transplant) in the ambient metal concentration between the 'recipient' and 'donor' transplant site , expressed as the concentration at the 'recipient' site divided by the concentration at the 'donor' site.

	zinc	lead
L.T. Transplant		
0061 05 0061 11	7	2
0061 05 0071 98	43	6
S.T. Transplant		
0061 05 0061 11	not available	3

5.43 Ratio diagrams

A simple method for determining whether there was any difference in the accumulation or loss of different metals by Scapania involves the use of ratio diagrams(Section 3.91). They allow the direct comparison between the relative concentrations of zinc and lead (expressed as Pb / (Pb + Zn).100= level of percentage lead (L.P.L.)).

A consistent pattern appears in the ratio diagrams (Figures 12 to 14). In reaches 0061 11 and 0071 98 the L.P.L. in the water fell to below 10% by Day 5 and it remained at this level for the rest of the period studied. The L.P.L. in the transplants which were moved into these reaches fell rapidly following transplantation, to attain a minimum value by Day 15. After Day 15 there was a slow recovery in the L.P.L. to approximately 40% (Scapania)and 50% (Chiloscyphus). The L.P.L. in the controls fell slowly over this period.

Zinc was accumulated by Scapania at a greater rate than lead (Section

5.42) during the initial period of the transplants (Tables 7 and 15). The high rate of zinc accumulation was probably at least partially a consequence of the difference in zinc concentrations between the reaches. If this was the sole determining factor, then the relative concentrations of zinc and lead in the transplants, would come to reflect those in the water of the 'recipient' site. This would appear to be happening until Day 15, as the L.P.L. in the transplants fell towards the L.P.L. in the water. After Day 15 the situation changed as the L.P.L. in the transplants increased. The accumulation rate of lead, therefore, has increased relative to that of zinc after Day 15. This change can not be explained in terms of the water chemistry and suggests that there exists an intrinsic difference between zinc and lead accumulation characteristic to Scapania.

Two possible explanations are forwarded to explain the change in the relative rates of zinc and lead accumulation.

i) The rate of lead accumulation is naturally greater than the rate of zinc accumulation. Due to the fact that the difference in ambient zinc concentrations between reaches was greater than the difference in lead concentrations, zinc accumulation initially appeared greater.

ii) The rate of zinc accumulation is either greater or the same as the rate of lead accumulation. The concentration to which lead can be accumulated by Scapania is proportionally greater, however, than that to which zinc can be accumulated.

It is the second explanation given above which seems most likely. Lead forms stronger bonds with organic matter than zinc (Ruhling and Tyler, 1970; Puckett et al., 1973). This would explain why lead normally has a higher enrichment ratio than zinc (Table 13). Zinc, because of its higher rate of accumulation and/or its lower enrichment ratio, would have a shorter response time to change (Section 5.4). Due to the behaviour of the controls in reach 0061 11, the response time for that reach can not be determined. In reach 0071 98, however, the change in the metal concentration in the controls was small and the response times can be determined. The response time of the 1cm fraction of Scapania was approximately 10 days for zinc and 60 days for lead.

In the transplants from reach 0061 11 to reach 0061 05, the L.P.L. in Scapania fell from approximately 50% to 35% and in Chiloscyphus from 70% to 50%. The L.P.L. in the transplants was by Day 58 similar to that of the water. It is possible therefore that there is no intrinsic difference in the rate of zinc and lead loss.

5.5 Differences between fractions

The 1-2cm fractions of Scapania and Chiloscyphus had a higher concentration of zinc and lead than the 1cm fractions. The difference in concentrations between the fractions varied but the concentration in the 1-2cm fraction could be up to four times greater than that in the 1cm fraction (Tables 25 to 29). The large differences possible between the fractions is of importance when considering the causes of variation in the enrichment ratio of Scapania (Section 5.1).

It is likely that the difference in the metal concentrations between fractions is related to some metabolic and/or morphological change occurring down the length of the stem. This would most likely be due to the increasing age of the material away from the tip. The rate of growth would, therefore, indirectly determine the increase of metal concentration down the stem. The rate of growth varies substantially from stem to stem (Table 40) and therefore the amount of new material in a 1cm fraction varies. This could be a major contributing factor to the amount of variation found in the metal concentration of the fractions and, as a consequence, in the enrichment ratios.

An increase in metal concentration down the length of the stem has been reported in other lotic macrophytes. Harding and Whitton (in press) reported finding increasing concentrations of zinc and lead as older 2cm fractions of Lemanea fluviatilis were taken. Lloyd (1977) reported that in bryophytes the zinc and lead concentrations increased down the stem from the tip. He suggested that it was the youngest material of Fontinalis antipyretica which exhibited the least variability and was the most reliable monitor of environmental metal concentrations. In this present study the 1cm fraction did exhibit less variation in metal concentrations than the 1-2cm fraction of Scapania.

Two possible explanations are forwarded to explain the difference in zinc and lead concentrations found between the fractions.

i) Scapania exhibits a continual net rate of accumulation, so that the concentration of metals in the tissue is related to the time over which the material has been exposed. Older bryophyte material would, therefore, have a higher zinc and lead concentration (Section 5.2).

ii) There is a difference in the rate of accumulation shown by the two fractions.

The rates of accumulation can be calculated from the change in metal concentrations following transplantation (Table 17). The rate for the initial period of the L.T. Transplant was calculated from Day 0 to Day 3 and from Day 1 to Day 3, due to the possibility of error in the figures for Day 0 (Section 5.41).

In the majority of cases the rate of accumulation of zinc and lead appeared to be greater in the 1-2cm fraction. A possible explanation for these results may lie in the fact that the 1-2cm fractions contain a larger percentage of dead material than the 1cm fraction. Living and dead organic materials both have a tendency to absorb trace elements (Sutcliffe and Baker, 1974). Both Pickering and Puia (1969) using Fontinalis antipyretica and Duncker (1974) using Scapania undulata showed that dead material took up zinc more readily than live.

5.6 Chiloscyphus

The reactions of Scapania and Chiloscyphus to transplantation were broadly similar. As far as can be judged from the limited

Table 17. Comparison of the rates of zinc and lead accumulation as shown by the 1cm and 1-2cm fractions in $\mu g\ g^{-1}\ d^{-1}$.

	Scapania				Chiloscyphus	
	0061 05 – 0061 11		0061 05 – 0071 98		0061 05 – 0061 11	
	1cm	1-2cm	1cm	1-2cm	1cm	1-2cm
L.T. Transplant						
Day0 to Day 3						
zinc	+ 178	+ 513	+ 1317	+ 2195	+ 223	+ 469
lead	+ 96	+ 47	+ 517	+ 677	+ 92	+ 83
Day 1 to Day 3						
zinc	+ 123	+ 479	- 534	+ 381		
lead	+ 76	+ 124	+ 230	+ 402		
S.T. Transplant						
Day 0 to Day 2						
zinc	+ 1100	+ 1800				
lead	+ 425	not available				

information, the response times were comparable and the rates of uptake and loss not significantly different. It was, however, noticeable that there were differences in the ratio diagrams. Chiloscyphus tended to have a higher level of percentage lead than Scapania (Figures 12 to 14).

Variation in the zinc and lead concentrations of replicate samples was greater in Chiloscyphus than in Scapania. The large standard deviations obtained, could, to a certain extent, be attributable to the life form of the bryophyte; the large number of branchings make fractionation difficult.

5.7 Suggestions for further research

In a report of this length it is not possible to come to any firm conclusions. It is, however, possible to clarify areas for further research.

The use of enrichment ratios to interpretate the concentration of zinc and lead found in Scapania has been a central theme of the discussion. The aim was to discover the causes of the variation in the enrichment ratios found for Scapania. Three factors were thought to contribute to this variation.

i) The increase in the zinc and lead concentration of the controls over the study period (Section 5.2). It is essential to discover whether this was peculiar to the conditions at the time and, if not, what were its causes.

ii) The response time of a monitor to change in the ambient metal concentration (Section 5.3). It was not possible to adequately determine the response time of Scapania to transplantation due to the behaviour of the controls, so that further transplant experiments are required merely to discover the time scale over which it operates. The response time is a consequence of both environmental and biotic factors. The degree to which these separate facts influence the response time should be determined. The rates of accumulation and loss are an important characteristic in this respect, and investigation into the relative rates of accumulation and loss of different heavy metals would yield useful results.

iii) The increase in metal concentration down the length of the bryophyte stem (Section 5.5). It is necessary to discover the cause of this phenomenon as it may well be related to the rate of growth. Considerable variation was discovered in the rate of growth within the same reach of river. This could introduce substantial variation into the assessment of the metal concentration of the bryophyte if based on fractions of a set length.

SUMMARY

i) *Scapania undulata* (L.) Dum. was investigated in relation to its use as a monitor of ambient zinc and lead concentrations.

ii) Clumps of *S. undulata* were transplanted between two reaches of the River Derwent and a tributary, Bolts Burn; the lead and zinc concentrations in the water at the three sites differed. Two transplant experiments were undertaken: a long term transplant lasting 58 days and a short term transplant lasting two days. Within each reach the native populations of *S. undulata* were sampled (controls).

iii) Clumps of *Chiloscyphus polyanthus* (L.) Corda var. *rivularis* (Schrad.) Nees. were also transplanted to act as a source of comparison.

iv) All transplanted bryophyte material displayed a change in the concentration of accumulated zinc and lead. As a consequence of this change, the difference between the metal concentrations of the transplants and those of the controls decreased. The rate at which this occurred varied from transplant to transplant.

v) During the long term transplant the concentration of zinc and lead in the water decreased or remained approximately level in all reaches, with the exception of reach 0061 11. In reach 0061 11 the concentration of zinc increased two fold.

vi) The control population of *S. undulata* reflected the fall in the aqueous lead concentration in reach 0061 05 and 0071 98. Most

of the other controls did not appear to monitor the change in the aqueous metal concentration; in most cases the concentration of zinc and lead in *S. undulata* and *C.* POLYANTHUS VAR. *rivularis* increased. Possible explanations for this phenomenom were discussed.

vii) The response time of *S. undulata* to the change in aqueous zinc and lead concentrations subsequent to transplantation was defined, and its significance discussed.

viii) The rate of accumulation and loss of metals was compared. Over the first three days the rate of zinc and lead loss appeared greater than their rate of accumulation. No conclusions were reached concerning these rates over the full 58 days.

ix) The different rates of accumulation and loss of zinc and lead were compared. The rate of zinc accumulation was greater than that of lead over the first three days. The change in aqueous metal concentration as a consequence of transplantation was greatest in the case of zinc. It was suggested, therefore, that the differences observed in rates of accumulation and loss could be a consequence of this.

x) Two fractions of the bryophyte stems were collected; these were the first two successive 1 cm lengths (1cm fraction and 1-2cm fraction). The 1-2cm fraction had a higher concentration of zinc and lead than the 1cm fraction. Possible explanations for this finding and its significance were discussed. The rate of accumulation of zinc and lead was generally found to be greater in the 1-2cm fraction.

xi) The reaction of *S. undulata* and *C.* POLYANTHUS VAR. *rivularis* to transplantation were broadly similar.

xii) An important characteristic of *S. undulata*, in relation to its use as a monitor, is the stability of the relationship between the aqueous metal concentration and the concentration accumulated. The enrichment ratio, which is the quantitative expression of this relationship, displayed substantial variation; having a coefficient of variance of up to 64% for lead and 83% for zinc. Possible causes of this variation were discussed. The major sources of variation were thought to be: the behaviour of the controls(vi); the length of the response time(vii); the difference between fractions (x).

APPENDIX A. Water samples collected by I.G. Burrows during spring survey (27 April - 1 May).

Table 18: Water samples from reach 0061 05

Table 19: Water samples from reach 0061 11

Table 20: Water samples from reach oo71 98

Table 21: Phosphate and fluoride concentrations in reach 0061 05

Table 22: Phosphate and fluoride concentrations in reach 0061 11

Table 18. Water samples from reach 0061 05 (mg l^{-1})

	SAMPLING DATES						
	26.04.79	27.04.79	28.04.79	29.04.79	01.05.79	Mean	Standard Deviation
Iron							
"Total"	0.63	0.46	0.4	0.43	0.53	0.49	0.09
"Filtered	0.56	0.40	0.34	0.33	0.41	0.41	0.09
Manganese							
"Total"	0.105	0.097	0.064	0.064	0.056	0.08	0.02
"Filtered"	0.108	0.092	0.09	0.056	0.067	0.08	0.02
Magnesium							
"Total"	1.74	2.0	1.87	2.00	2.5	2.02	0.2
"Filtered"	1.72	1.97	1.88	1.94	2.42	1.99	0.36
Calcium							
"Total"	6.29	7.55	7.16	7.61	9.61	7.6	1.22
"Filtered"	6.25	7.31	7.25	7.46	9.35	7.52	1.13
Potassium							
"Total"	1.12	1.16	1.25	1.19	1.42	1.23	0.12
"Filtered"	1.06	1.18	1.19	1.14	1.40	1.19	0.13
Sodium							
"Total"	4.0	4.3	4.5	4.0	5.9	4.54	0.79
"Filtered"	4.3	4.6	4.5	4.4	5.5	4.6	0.48
Zinc							
"Total"	0.022	0.015	0.024	0.017	0.032	0.022	0.007
"Filtered"	0.020	0.014	0.015	0.013	0.021	0.017	0.004
Lead							
"Total"	0.01	0.007	0.006	0.005	0.007	0.007	0.002
"Filtered"	0.008	0.005	0.006	0.003	0.014	0.007	0.004

Table 19. Water samples from reach 0061 11 (mg l^{-1})

	SAMPLING DATES						
	26.04.79	27.04.79	28.04.79	29.04.79	01.05.79	Mean	Standard Deviation
Iron							
"Total"	0.53	0.37	0.36	0.31	0.30	0.37	0.09
"Fitered"	0.49	0.40	0.26	0.25	0.23	0.33	0.11
Manganese							
"Total"	0.116	0.12	0.102	0.078	0.091	0.10	0.02
"Filtered"	0.116	0.117	0.104	0.08	0.099	0.103	0.02
Magnesium							
"Total"	2.27	2.75	2.69	2.71	3.36	2.76	1.66
"Filtered"	2.23	2.74	2.61	2.63	3.36	2.72	0.39
Calcium							
"Total"	8.96	11.5	11.4	11.3	15.8	11.79	2.48
"Filtered"	8.95	11.5	11.4	10.9	15.2	11.59	2.27
Potassium							
"Total"	1.42	1.74	1.82	1.65	2.14	1.75	0.26
"Filtered"	1.5[illegible]	1.77	1.87	1.63	2.18	1.79	0.26
Sodium							
"Total"	5.6	7.0	7.2	6.4	9.1	7.06	1.30
"Filtered"	5.7	7.2	7.4	6.1	9.3	7.15	1.41
Zinc							
"Total"	0.126	0.149	0.172	0.140	0.211	0.16	0.03
"Filtered"	0.126	0.152	0.167	0.132	0.203	0.16	0.03
Lead							
"Total"	0.029	0.038	0.021	0.018	0.026	0.026	0.007
"Filtered"	0.027	0.024	0.015	0.013	0.017	0.019	0.006

Table 20. Water samples from reach 0071 98 (mg l^{-1})

	SAMPLING DATES						
	26.04.79	27.04.79	28.04.79	29.04.79	01.05.79	Mean	Standard Deviation
Iron							
"Total"	0.16	0.12	0.12	0.20	0.12	0.14	0.04
"Filtered"	0.08	0.07	0.07	0.07	0.07	0.07	0.004
Manganese							
"Total"	0.22	0.216	0.282	0.15	0.323	0.24	0.07
"Filtered	0.214	0.21	0.265	0.148	0.32	0.23	0.06
Magnesium							
"Total"	4.5	5.0	5.2	5.3	5.9	5.18	0.5
"Filtered"	4.5	5.0	5.1	5.1	5.7	5.08	0.4
Calcium							
"Total"	22.5	24.9	26.4	26.8	29.7	26.06	2.64
"Filtered	22.4	24.5	26.4	26.4	28.9	25.7	2.43
Potassium							
"Total"	3.29	3.74	3.96	3.94	4.42	3.99	0.25
"Filtered"	3.25	3.73	3.96	3.91	4.29	3.83	0.38
Sodium							
"Total"	14.7	17.3	18.6	16.5	20.2	17.5	2.08
"Filtered"	15.1	17.2	18.2	16.9	19.5	17.38	1.63
Zinc							
"Total"	0.77	0.81	0.84	0.91	0.99	0.86	0.09
"Filtered"	0.75	0.78	0.78	0.86	0.93	0.82	0.07
Lead							
"Total"	0.145	0.103	0.064	0.096	0.058	0.09	0.03
"Filtered"	0.087	0.092	0.052	0.066	0.036	0.067	0.02

Table 21. Phosphate and fluoride concentrations in reach 0061 05 (mg l^{-1})

	SAMPLING DATES				Mean	Standard Deviation
	26.04.69	27.04.79	28.04.79	29.04.79		
Phosphate (PO_4-P)	0.02	0.021	0.023	0.020	0.021	0.001
Fluoride	0.42	0.40	0.39	0.39	0.4	0.01
	08 79	08.79	08.79			
Phosphate (PO_4-P)	0.024	0.023	0.021	0.024	0.02	0.001
Fluoride	0.74	0.72	0.72	0.72	0.73	0.01

Table 22. Phosphate and fuoride concentrations in reach 0061 11 (mg l^{-1})

	SAMPLING DATES				Mean	Standard Deviation
	26.04.79	27.04.79	28.04.79	29.04.79		
Phosphate (PO_4-P)	0.017	0.019	0.021	0.018	0.019	0.002
Fluoride	0.75	0.76	0.76	0.74	0.75	0.009
	08.79	08.79	08.79			
Phosphate (PO_4-P)	0.02	0.02	0.22	0.019	0.02	0.001
Fluoride	1.80	1.85	1.70	1.90	1.8	0.08

APPENDIX B. Water chemistry for long term transplant

Table 23: Zinc concentrations (mg l^{-1})

Table 24: Lead concentrations (mg l^{-1})

<u>Table 23</u>. Zinc concentrations of water at transplant sites (mg l^{-1})

	"TOTAL"	"FILTERED"
22.05.79		
0061 05	0.04	0.03
0061 11	0.21	0.20
0071 98	1.30	1.28
23.05.79		
0061 05	0.06	0.06
0061 11	0.30	0.14
0071 98	0.96	0.85
25.05.79		
0061 05	0.04	0.04
0061 11	0.28	0.25
0071 98	1.24	1.14
29.05.79		
0061 05	0.03	0.027
0061 11	0.47	0.43
0071 98	2.14	1.98
06.06.79		
0061 05	0.02	0.03
0061 11	0.28	0.28
0071 98	1.00	0.89
22.06.79		
0061 05	0.023	0.018
0061 11	0.23	0.25
0071 98	0.87	0.87
19.07.79		
0061 05	0.012	0.016
0061 11	0.56	0.58
0071 98	0.81	0.84

Table 24. Lead concentrations of water at transplant sites. (mg l^{-1})

	"TOTAL"	"FILTERED"
22.05.79		
0061 03	0.01	0.01
0061 11	0.09	0.023
0071 97		0.058
23.05.79		
0061 03	0.05	0.031
0061 11	0.17	0.055
0071 97	1.48	0.12
25.05.79		
0061 03	0.01	0.01
0061 11	0.03	0.023
0071 97	0.062	0.058
29.05.79		
0061 03	0.02	0.01
0061 11	0.037	0.035
0071 97	0.10	0.07
6.06.79		
0061 03	0.01	0.01
0061 11	0.034	0.019
0071 97	0.05	0.037
22.06.79		
0061 03	0.005	0.005
0061 11	0.032	0.030
0071 97	0.074	
19.07.79		
0061 03	0.006	0.006
0061 11	0.023	0.024
0071 97	0.059	0.048

<u>APPENDIX [illegible]</u>. Concentration of zinc and lead in *Scapania undulata* during the long term transplant ($\mu g\ g^{-1}$)

Table 25: Concentration of zinc and lead in *Scapania undulata* for reach 0061 05

Table 26: Concentration of zinc and lead in *Scapania undulata* for reach 0061 11

Table 27 Concentration of zinc and lead in *Scapania undulata* for reach 0071 98

Table 28 Concentration of zinc and lead in *Chiloscyphus polyanthus* var. *rivularis* for reach 0061 05

Table 29 Concentration of zinc and lead in *Chiloscyphus polyanthus* var. *rivularis* for reach 0061 11

Table 25. Concentration of zinc and lead in Scapania undulata ($\mu g\ g^{-1}$) during the long term transplant (reach 0061 05)

	Fraction	Pb levels ($\mu g\ g^{-1}$)			No. of Samples	Zn levels ($\mu g\ g^{-1}$)		
			S.D.	C.V.			S.D.	C.V.
22.05.79	1 cm	1678	297	17	4	1674	559	33
(Day 0)	1-2 cm	4721	609	13	5	3659	1708	47
23.05.79	1 cm	2933			1	2755		
(Day 1)	1-2 cm	4361			1	4855		
	controls							
	1 cm	144			1	185		
	1-2 cm	296			1	237		
25.05.79	1 cm	1822	379	21	4	1623	603	37
(Day 3)	1-2 cm	4427	529	12	4	3352	1103	33
	controls							
	1 cm	253			2	288		
	1-2 cm	513			2	710		
29.05.79	1 cm	1117			3	1150		
(Day 7)	1-2 cm	3846			1	4936		
	controls							
	1 cm	198			2	199		
	1-2 cm	338			2	509		
6.06.79	1 cm	1121	171	15	5	1282	391	31
(Day 15)	1-2 cm	3568	1279	15	4	4072	1377	34
	controls							
	1 cm	369			2	609		
	1-2 cm	500			2	776		
22.06.79	1 cm	6799	71	10	4	9998	117	12
(Day 31)	1-2 cm	3357	217	6	4	27898	9715	35
	controls	293			2			
	1-2 cm	379			2	2636		
19.07.79	1 cm	239	41	17	5	500	37	7
(Day 58)	1-2 cm	1091	373		5	1379		
	controls							
	1 cm	1287	27	21%	5	3998	34	9
	1-2 cm	249	58	24	5	952	151	16

Table 26. Concentration of zinc and lead in Scapania undulata ($\mu g\ g^{-1}$) during the long term transplant (reach 0061 11)

	Fraction	Pb levels ($\mu g\ g^{-1}$)	S.D.	C.V.	No. of samples	Zn levels ($\mu g\ g^{-1}$)	S.D.	C.V.
22.05.79	1 cm	194	7.9	4	4	249	38	15
(Day 0)	1-2 cm	455	8.7	2	4			
23.05.79	1 cm	333			1	538		
(Day 1)	1-2 cm	379			1	1049		
	control							
	1 cm				1	4760		
	1-2 cm				1	6060		
25.05.79	1 cm	484	70	14	5	783	216	28
(Day 3)	1-2 cm	627	26	4	4	2006	123	6
	control							
	1 cm	1719			2	2876		
	1-2 cm	4042			2	5394		
29.05.79	1 cm	611	48	8	5	1894	123	6.5
(Day 7)	1-2 cm	940	204	22	5	3063	385	13
	control							
	1 cm	2033			2	1953		
	1-2 cm	4711			2	3541		
6.06.79	1 cm	735	137	19	5	3073	179	5.8
(Day 15)	1-2 cm	872	49	6	5	4444	435	9.8
	control							
	1 cm	1779			2	4222		
	1-2 cm	5095			2	8775		
22.06.79	1 cm	1862	397	21	4	3081	258	8.4
(Day 31)	1-2 cm	2020	442	22	4	4778	651	14
	control							
	1 cm	2659			2	5577		
	1-2 cm	5719			2	14040		
19.07.79	1 cm	4152	363	9	5	6507	385	5.9
(Day 58)	1-2 cm	4932	394	8	5	9265	1007	11
	control							
	1 cm	5583	1478	27	5	8561	1232	14
	1-2 cm	9143	428	3	5	24429	4158	30

Table 27. Concentration of zinc and lead in Scapania undulata ($\mu g\ g^{-1}$) during the long term transplant (reach 0071 98)

	Fraction	Pb levels ($\mu g\ g^{-1}$)	S.D.	C.V.	No. of samples	Zn levels ($\mu g\ g^{-1}$)	S.D.	C.V.
22.0.579	1 cm	211	76	36	5	235	80	34
(Day 0)	1-2 cm	471	58	12	5	383	71	19
23.05.79	1 cm	1302			1	5254		
(Day 1)	1-2 cm	1697			1	6103		
	control							
	1 cm	11385			1			
	1-2 cm	13768			1			
25.05.79	1 cm	17612	268	15	5	4186	1337	32
(Day 3)	1-2 cm	2501	487	19	5	6968	1437	21
	control							
	1 cm	10418			2	6865		
	1-2 cm	14181			2	7101		
29.05.79	1 cm	3246	385	12	5	9217	775	8
(Day 7)	1-2 cm	4272	474	11	5	11393	2494	22
	control							
	1 cm	13636			2	7022		
	1-2 cm	19847			2	5945		
6.06.79	1 cm	2960	94	32	4	9870	1100	
(Day 15)	1-2 cm	5583	975	18	4	14428	3440	24
	control							
	1 cm	87098			2			
	1-2 cm	15353			2			
22.06.79	1 cm	6333	842	13	5	11514	13317	11
(Day 31)	1-2 cm	7965	1305	10	5	12784	2421	18
	control							
	1 cm	10200			2	8977		
	1-2 cm	13285			2	8854		
19.07.79	1 cm	5602	867	13	5	11342	2174	19
	1-2 cm	10137	819	8	5	14370	599	4
	control							
	1 cm	9262	2560	28	2	8806	567	6
	1-2 cm	14558	2628	18	2	7419	235	3

Table 28. Concentration of zinc and lead in Chiloscyphus polyanthus var. rivularis ($\mu g\ g^{-1}$) during the Long term transplant (reach 0061 05)

	Fraction	Pb levels ($\mu g\ g^{-1}$)	S.D.	C.V.	No. of samples	Zn levels ($\mu g\ g^{-1}$)	S.D.	C.V.
22.05.79	1 cm	2257			1	929		
(Day 0)	1-2 cm	5719			1	2247		
23.05.79	1 cm							
(Day 1)	1-2 cm							
	control							
	1 cm							
	1-2 cm							
25.05.79	1 cm	1799			2	1477		
(Day 3)	1-2 cm	5667			2	2378		
	control							
	1 cm							
	1-2 cm							
29.05.79	1 cm	4867			2	17167		
(Day 7)	1-2 cm	6496			2	1974		
	control							
	1 cm							
	1-2 cm							
6.06.79	1 cm	1521	1046	69	5	1001	334	33
(Day 15)	1-2 cm	3970			5	1893	1200	
	control							
	1 cm							
	1-2 cm							
22.06.79	1 cm	862	213	25	5	842	83	9.9
(Day 31)	1-2 cm	4574	998	23	5	15518	1668	11
	control							
	1 cm							
	1-2 cm							
19.07.79	1 cm	308.5	81	26	5	358	203.9	56.9
(Day 58)	1-2 cm	588	194	33	5	778	320	41
	control							
	1 cm	254	106	42	5	324	60	19
	1-2 cm	663	35.7	54	5	788	320	41

Table 29. Concentration of zinc and lead in Chiloscyphus polyanthus var. rivularis (reach 0061 11)

	Fraction	Pb level ($\mu g\ g^{-1}$)	S.D.	C.V.	No. of samples	Zn levels ($\mu g\ g^{-1}$)	S.D.	C.V.
22.05.79	1 cm	120			1	187		
(Day (0)	1-2 cm	319			1	350		
23.05.79	1 cm							
(Day 1)	1-2 cm							
	control							
	1 cm							
	1-2 cm							
25.05.79	1 cm	395			2	116		
(Day 3)	1-2 cm	570			2	1757		
	control							
	1 cm							
	1-2 cm							
29.05.79	1 cm	511			2	1897		
(Day 7)	1-2 cm	741			2	2604		
	control							
	1 cm							
	1-2 cm							
6.06.79	1 cm	712	64	9	5	2932	264	9
(Day 15)	1-2 cm	925	180	20	5	4090	915	22
	control							
	1 cm							
	1-2 cm							
22.06.79	1 cm	3336	1183	35	5	3493	402	12
(Day 31)	1-2 cm	3479	2213	64	5	5550	1170	21
	control							
	1 cm							
	1-2 cm							
19.07.79	1 cm	3899	304	8	5	6241	555	9
(Day 58)	1-2 cm	47896	1135	24	5	7135	1321	19
	control							
	1 cm	3029	597	20	5	6425	592	9
	1-2 cm	4615	847	18	5	11947	553	5

<u>APPENDIX D</u>. Ratio diagram data expressed as level of percentage lead (Pb / (Pb + Zn). 100). and enrichment ratios.

Table 30: L.P.L. in <u>Scapania</u> <u>undulata</u> and <u>Chiloscyphus</u> <u>polyanthus</u> var. <u>rivularis</u> during the long term transplant

Table 31: L.P.L in the water of the transplant sites.

Table 32: L.P.L in <u>Scapania</u> <u>undulata</u> during the short term transplant.

Table 33: Enrichment ratios of zinc displayed by the control population of <u>Scapania</u> <u>undulata</u>

Table 34: Enrichment ratios of lead displayed by the control population of <u>Scapania</u> <u>undulata</u>

Table 30. Level of percentage lead in Scapania undulata and Chiloscyphus polyanthus var. rivularis during the long term transplant.

		Scapania undulata transplant						Chiloscyphus transplant			
		0061 11→0061 05		0061 05→0061 11		0061 05→0071 98		0061 11→0061 05		0061 05→0061 11	
	Fraction	(Pb/Pb+Zn)x100		(Pb/Pb+Zn)x100		(Pb/Pb+Zn)x100		(Pb/Pb+Zn)x100		(Pb/Pb+Zn)x100	
		Scapania		Scapania		Scapania		Chiloscyphus		Chiloscyphus	
			S.D.		S.D.		S.D.		S.D.		S.D.
22.05.79	1 cm	50%	4.8	44%	4.4	46%	3.3	65%		39%	
(Day 0)	1-2 cm	57%	13.0	49%	8.3	55%	9.1	72%		48%	
23.05.79	1 cm	52%		38%		22%	0.7				
(Day 1)	1-2 cm	47%		27%		22%	0.7				
	control										
	1 cm	44%				65%					
	1-2 cm	56%				72%					
25.05.79	1 cm	53%	13.0	37%	1.7	30%	2.0	62%		47%	
(Day 3)	1-2 cm	57%	8.7	23%	4.23	26%	4.0	70%		42%	
	control										
	1 cm	47%		37%		60%					
	1-2 cm	42%		43%		67%					
29.05.79	1 cm	48%	0.5	24%	0.5	26%	2.6	65%		21%	
(Day 7)	1-2 cm	47%	0.7	23%	0.5	27%	2.4	77%		22%	
	control										
	1 cm	49%		51%		65%					
	1-2 cm	40%		57%		79%					
6.06.79	1 cm	46%	2.3	19%	0.3	23%	2.3	53%	1.9	19%	1.6
(Day 15)	1-2 cm	47%	6.3	16%	0.4	28%	2.4	58%	±17.0	18%	2.0
	control										
	1 cm	38%		29%		50%					
	1-2 cm	38%		37%		62%					
22.06.79	1 cm	41%	3.3	37%	2.1	35%	7.1	50%	0.7	47%	2.6
(Day 31)	1-2 cm	53%	3.2	35%	2.0	38%	12.5	74%	12.5	35%	1.8
	control										
	1 cm			32%		63%					
	1-2 cm			28%		67%					
19.07.79	1 cm	32%	2.7	40%	7.7	37%	8.3	45%	1.4	38%	4.3
(Day 58)	1-2 cm	45%	9.6	35%	11.0	41%	7.9	42%	2.0	39%	2.5
	control										
	1 cm	24%	2.5	39%	3.6	49%	2.9	38%	0.9	32%	4.2
	1-2 cm	21%		26%	1.8	66%	11.5	37%	1.0	28%	2.6

Table 31. Level of percentage lead in the water of the transplant sites

	"TOTAL"	"FILTERED"
22.05.79		
05	20%	25%
11	30%	49%
98		4%
23.05.79		
05	45%	34%
11	36%	28%
98	61%	12%
25.05.79		
05	20%	20%
11	10%	8%
98	5%	5%
29.05.79		
05	40%	27%
11	7%	8%
98	4%	3%
6.06.79		
05	33%	25%
11	10%	6%
98		
22.06.79		
05	17%	22%
11	6%	11%
98		
19.07.79		
05	33%	27%
11	4%	4%
98	7%	5%

Table 32. Level of percentage lead in <u>Scapania</u> <u>undulata</u> during the long term transplant.

hours after transplant	time	lead/zinc ratios				
		0061 05 control	0061 11 to 0061 05	0061 11 water	0061 11 control	0061 05 to 0061 11
-2	8.45	17.3	33.0	5.3	33.6	17.3
0	10.45					
1	11.45	23.0	28.2	5.3	32.6	23.7
2	12.45	24.0	32.6	9.3	29.7	15.8
3	13.45	29.0	35.4	5.7	34.7	17.2
4	14.45	24.9	34.8	6.2	35.3	24.5
5	15.45	38.5	30.7	7.8	42.6	17.6
6	16.45	26.7	35.5	7.6	35.3	17.8
7	17.45	24.2	34.1	6.7	34.1	23.6
8	18.45	27.7	32.1	8.3	35.7	28.0
9	19.45	13.3	37.4	5.7	35.7	14.4
11	21.45	13.2		6.3	34.0	
13	23.45	22.9	34.2	5.4	33.9	19.5
15	1.45	21.0	32.5	5.6	36.5	20.9
17	3.45	31.5	41.0	5.1	32.7	15.2
19	5.45	35.8	34.9	4.7	33.8	15.5
21	7.45	19.4	33.7	5.7	31.2	23.8
23	9.45	19.4	38.4	6.3	31.6	18.8
26	12.45	28.9	37.9	8.1	32.5	21.3
29	15.45	22.3	34.7	9.9	32.7	23.7
31	18.45	27.1	38.2	6.3	34.5	17.8
34	21.45	21.3	36.2	7.4	37.1	13.9
48	10.45		41.1	5.9		25.8
		average lead/zinc ratios				
		24.6	34.9	6.6	34.8	19.5

Table 33. Enrichment ratios of zinc displayed by the control population of *Scapania undulata*.

	water(mg l^{-1})	*Scapania*($\mu g\ g^{-1}$)		enrichment ratio	
	'filtered'	1cm	1-2cm	1cm	1-2cm
reach 0061 05					
Day 0	0.03	235	383	7800	12800
Day 1	0.06	185	237	3100	4000
Day 3	0.04	288	710	7200	17800
Day 7	0.027	199	509	7400	18900
Day 15	0.03	608	776	20300	25900
Day 58	0.016	399	952	6700	59500
reach 0061 11					
Day 0	0.2	929	2247	4700	23200
Day 1	0.14	4760	6060	3400	43300
Day 3	0.25	2876	5394	11500	21600
Day 7	0.43	1953	3541	4600	8200
Day 15	0.28	4222	8775	15100	31300
Day 31	0.25	5577	14040	22300	56200
Day 58	0.58	8561	24429	14800	42100
reach 0071 98					
Day 3	1.14	6865	7101	6000	6200
Day 7	1.98	7022	5945	3600	3000
Day 31	0.87	8977	8854	10300	10200
Day 58	0.84	8806	7419	10500	8800

Table 34. Enrichment ratios of lead displayed by the control population of *Scapania undulata*.

	water(mg l^{-1}) 'filtered'	*Scapania*(µg g^{-1}) 1cm	1-2cm	enrichment ratio 1cm	1-2cm
reach 0061 05					
Day 0	0.01	194	455	19400	15500
Day 1	0.031	144	296	4700	9600
Day 3	0.01	253	513	25300	51300
Day 7	0.01	198	338	19800	33800
Day 15	0.01	369	500	36900	50000
Day 31	0.005	293	379	58600	75800
Day 58	0.005	128	249	21300	41500
reach 0061 11					
Day 0	0.023	1678	4721	73000	205300
Day 3	0.033	1719	4042	74700	122500
Day 7	0.035	2033	4711	58100	134600
Day 15	0.019	1779	5095	93600	268200
Day 31	0.03	2659	5719	88600	190600
Day 58	0.024	5583	9143	23300	381000
reach 0071 98					
Day 1	0.12	11385	13768	94900	114700
Day 3	0.058	10418	14181	179600	244500
Day 7	0.07	13636	19847	194800	283500
Day 15	0.037	8709	15353	235400	414900
Day 58	0.048	9262	14558	193000	303300

APPENDIX E. Short term transplant

Table 35. Physico-chemical conditions during the short term transplant for reach 0061 05

Hours after transplant	Time	pH	T(^{o}C)	ZINC (mg l^{-}) "Total"	ZINC (mg l^{-}) "Filtered"	LEAD (mg l^{-}) "Total"	LEAD (mg l^{-}) "Filtered"
-2	8.45	7.25	10.5			.005	.012
0	10.45						
1	11.45	7.6	11.5			.007	
2	12.45	7.35	12.0			.004	
3	13.45	7.35	12.5			.004	
4	14.45	7.35	12.75			.008	
5	15.45	7.35	13.5			.004	
6	16.45	7.35	14.0			.003	
7	17.45	7.35	14.5			.001	
8	18.45	7.3	14.0			.001	
9	19.45	7.3	14.0			.003	
11	21.45	7.3	13.5			.001	
13	23.45	7.25	13.0			.002	
15	1.45	7.1	12.5			.001	
17	3.45	7.1	11.0			.001	
19	5.45	7.1	10.5			.001	
21	7.45	7.35	11.0			.001	
23	9.45	7.35	11.5			.001	
26	12.45	7.3	12.5			.001	
29	15.45	7.5	14.5			.001	
31	18.45	7.3	15.0			.001	
34	21.45	7.3	11.75			.005	
48	10.45	7.3	11.75			.005	

Table 36. Physico-chemical conditions during the short term transplant for reach 0061 11.

Hours after transplant	Time	pH	T(°C)	ZINC ($mg\ l^{-}$) "Total"	ZINC ($mg\ l^{-}$) "Filtered"	LEAD ($mg\ l^{-}$) "Total"	LEAD ($mg\ l^{-}$) "Filtered"
-2	8.45	7.9	10.5	.42	.41	.0025	.019
0	10.45						
1	11.45	7.9	12.5	.39		.022	
2	12.45	8.2	13.5	.36		.037	
3	13.45	8.2	14.0	.36		.022	
4	14.45	8.2	14.5	.35		.023	
5	15.45	8.2	15.0	.33		.028	
6	16.45	8.1	15.0	.34		.028	
7	17.45	8.0	15.0	.36		.026	
8	18.45	8.0	15.0	.34		.031	
9	19.45	8.0	15.0	.36		.022	
11	21.45	7.65	14.5	.37		.025	
13	23.45	7.65	13.5	.40		.023	
15	1.45	7.4	12.75	.40		.024	
17	3.45	7.6	12.0	.41		.022	
19	5.45	7.75	11.5	.39		.019	
21	7.45	7.65	12.0	.36		.022	
23	9.45	7.45	12.5	.37		.025	
26	12.45	7.9	14.5	.32		.028	
29	15.45	7.95	16.0	.31		.034	
31	18.45	7.9	16.0	.34		.023	
34	21.45	7.9	15.0	.34		.027	
48	10.45	7.9	12.0	.35	.38	.022	.015

Table37. Concentration of zinc and lead in *Scapania undulata* ($\mu g\ g^{-1}$) during the short term transplant (reach 0061 05)

Hours after transplant	Time	LEAD (1 cm) 0061 05	LEAD (1 cm) 0061 11 to 0061 05	ZINC (1 cm) 0061 05	ZINC (1 cm) 0061 11 to 0061 05
-2	8.45	145.0±22	4895±241	694±114	
0	10.45				
1	11.45	269	4044	876	10294
2	12.45		3986	720	8213
3	13.45	274	4583	858	8333
4	14.45	278	4145	675	7772
5	15.45	267	2995	760	6770
6	16.45	287	6433	459	11696
7	17.45	242	3333	665	6428
8	18.45	203	4359	636	9231
9	19.45	302	3916	788	6549
11	21.45	261		1023	
13	23.45	155	3677	1013	7088
15	1.45	208	3514	699	7297
17	3.45	146	4972	547	7131
19	5.45	276	3645	599	6771
21	7.45	362	29994	649	5898
23	9.45	185	3736	769	5977
26	12.45	211	5208	879	8542
29	15.45	301	3163	739	5964
31	18.45	214	3459	746	5598
34	21.45	314	3935	844	6944
48	10.45		3512±449		5039±2826
		LEAD (1-2 cm)		ZINC (1-2 cm)	
-2	8.45	529±217	5388±251	1189±142	
48	10.45		6740±507		15896±2853

Table 38. Concentration of zinc and lead in Scapania undulata during short term transplant (reach 0061 11)

Hours after transplant	Time	LEAD (1 cm) 0061 11	LEAD (1 cm) 0061 05 to 0061 11	ZINC (1 cm) 0061 11	ZINC (1 cm) 0061 05 to 0061 11
-2	8.45	4895±241	145±22	9644	
0	10.45				
1	11.45	4459	283	9236	911
2	12.45	2794	255	6618	1352
3	13.45	4538	322	8478	1555
4	14.45	4223	484	7736	1487
5	15.45	3571	322	4809	1503
6	16.45	2857	361	5247	1667
7	17.45	2515	483	4853	1567
8	18.45	3417	449	6149	1154
9	19.45	2420	347	4362	2056
11	21.45	5323		10323	2558
13	23.45	4695	354	9137	1463
15	1.45	5220	572	9066	2152
17	3.45	5509	552	11299	3083
19	5.45	3966	489	7758	2654
21	7.45	4899	859	10811	2744
23	9.45	5316	625	11494	2694
26	12.45	4452	593	9246	2190
29	15.45	4750	948	9750	3049
31	18 45	5176	896	9848	4127
34	21 45	6210	1040	10509	6423
48	10 45		1015±62		2916±3105
		LEAD (1-2 cm)		ZINC (1-2 cm)	
12	8 45	5388±251	530±217	12823±341	
48	10 45		975±135		4855±401

APPENDIX F. Computer Programs.

Program 1. Calculation of levels in $\mu g\ g^{-1}$ from Wt. AAS readings.

Program 2. Calculation of means, standard deviations and coefficients of variance.

Program 3. Ghost program for graph drawing.

PROGRAM 1.

```
      DIMENSION CY(5),CZ(5)
      DO 14 I=1,5
14    READ(4,3)CY(I),CZ(I)
4     DO 13 N=1,6000
      READ(5,100,END=200)X,Y,IP,Z
      Z=((Z+CZ(IP))/X)*25000
      Y=((Y+CY(IP))/X)*25000
8     WRITE(6,99)X,Y,CY(IP),Z,CZ(IP)
13    CONTINUE

00    FORMAT (2(F5.2),I2,F5.2)
3     FORMAT (2(F5.2))
99    FORMAT (1H , 5(1X, G10.3))
```

PROGRAM 2.

```
      DIMENSION DATA(455)
      DIMENSION SAV(67)
      DIMENSION AV(67)
      DIMENSION IND(455)
      DIMENSION SD(67)
      DIMENSION CV(67)
      INTEGER COUNT
      DO 14 I=1,455
      READ(5,900)DATA( ),IND(I)
900   FORMAT(21X,F10.2 I5)
 14   CONTINUE
      K=1
      AV(1)=0
      COUNT=0
      DO 107 I=1,455
      IF(IND(I).EQ.999)GO TO 108
      IF(IND(I).NE.K) GO TO 100
      AV(K)=AV(K)+DATA I)
      COUNT=COUNT+1
      GO TO 107
100   AV(K)=AV(K)/COUN
      WRITE(6,17)AV(K) K
      K=IND(I)
      AV(K)=DATA(I)
      COUNT=1
108   WRITE(6,109)DATA(I),IND(I)
107   CONTINUE
 17   FORMAT(1H0, F10.2,1X,I4)
109   FORMAT(1H0,F10.3,I4)
      K=1
      SAV(K)=0
      COUNT=0
      DO 260 I=1,455
      IF(IND(I).EQ.999) GO TO 260
      IF(IND(I).NE.K) GO TO 200
      SAV(K)=SAV(K)+(DATA(I)-AV(K))**2
      COUNT=COUNT+1
      GO TO 260
200   SD(K)= SAV(K)/(COUNT-1)
      SD(K)=SQRT(SD(K))
      WRITE(6,17)SD(K),IND(I)
      K=IND(I)
      SAV(K)=(DATA(I)-AV(K))**2
      COUNT=1
260   CONTINUE
      SD(67)=999
      AV(67)=999
      AV(53)=999
      SD(53)=999
      SD(52)=999
      AV(52)=999
      SD(43)=999
      AV(43)=999
      DO 75 I=1,67
      CV(I)=SD(I)/AV(I)*100
      WRITE(6,191)SD(I),AV(I),CV(I)
 75   CONTINUE
191   FORMAT(1H0, 3F10.2)
      STOP
      END
```

PROGRAM 3.

```
      DIMENSION X(100),Y(100),S(100),TITLE(20),XAXIS(10),YAXIS(10)
      DIMENSION Z(100),W(100)
      CALL PSPACE (0.1,   0.9, 0.1,      0.7)
      CALL MAP    (0.0,  60.0, 0.0, 2.5)
      CALL AXES
      CALL BORDER
      CALL CTRSET(4)
      READ (5,100) TITLE
      READ(5,103)XAXIS
      READ(5,104)YAXIS
      READ (5,101) NL
      DO 30 IL=1,NL
      READ (5,101) NP
      DO 10 IP=1,NP
      READ (5,102) X(IP),Y(IP),S(IP)
      W(IP)=Y(IP)-S(IP)
      Z(IP)=Y(IP)+S(IP)
   10 CONTINUE
      CALL PTPLOT (X,Y,1,NP,40+IL)
      DO 50 IP=1,NP
      IF(S(IP).EQ.0.0) GO TO 50
      CALL PLOTNC (X(IP),Z(IP),44)
      CALL PLOTNC (X(IP),W(IP),44)
   50 CONTINUE
      DO 70 IP=1,NP
      CALL POSITN(X(IP),W(IP))
      CALL JOIN(X(IP),Z(IP))
   70 CONTINUE
   30 CONTINUE
      CALL PSPACE (0.0,1.0,0.0,.5)
      CALL MAP    (0.0,1.0,0.0,1.0)
      CALL CTRSET(1)
      CALL PLOTCS (0.1,0.92,TITLE,80)
      CALL PLOTCS(0.2,0.05,XAXIS,40)
      CALL CTRORI(1.0)
      CALL PLOTCS(0.02,0.2,YAXIS,40)
      CALL CTRORI(0.0)
      CALL GREND
      STOP
  100 FORMAT (20A4)
  103 FORMAT(10A4)
  104 FORMAT(10A4)
  101 FORMAT (I3)
  102 FORMAT (F10.0,F10.3,F10.0)
      END
```

APPENDIX G. Growth experiment

Table 39: length of stem of Scapania undulata when harvested

Table 40: Increase in length of stem in mm (Day 24)

Table 41: Increase in length of stem in mm (Day 51)

Table 39. Growth experiment; lengthof delimited stem when harvested (mm). Startei on the 29 May.

No. Of days after transplant	0061 05	0061 11 to 0061 05	0061 11	0061 05 to 0061 11	0071 98	0061 05 to 0071 98
Day 0						
stem length delimited at	5	5	5	5	5	5
Day 24						
mean	6.4	6.2	6.14	7.4	6.15	5.6
max.	9	8	8	10	7	7
S.D.	1.2	1.1	1.2	1.6	0.8	0.75
No.	24	20	20	25	20	20
Day 51						
mean	8	9.1	7.4	7.6	7.3	6.3
max.	11	14	10	12	10	9
S.D.	2.1	2.3	1.6	3.2	1.3	1.2
No.	15	11	16	5	29	20

Table 40. Growth experiment; increase in length in mm (original length 5 mm).

	0061 05	0061 11 to 0061 05	0061 11	0061 05 to 0061 11	0071 98	0061 05 to 0071 98
Day 24						
	3	3	0	3	0	0
	0	1	3	5	2	1
	1	2	0	2	2	2
	1	3	0	2	1	1
	2	3	3	3	3	1
	4	1	3	2	1	0
	3	2	0	3	2	0
	3	2	0	1	1	0
	2	1	0	2	1	0
	2	0	2	4	2	0
	1	0	1	2	1	2
	1	1	2	1	1	0
	0	2	1	0	1	1
	1	0	1	5	2	0
	0	0	2	1	0	2
	0	2	3	1	0	1
	2	1	0	2	0	0
	2	0	3	0	0	0
	2	0	0	3	2	1
	0	0	0	5	1	0
	3			4		
	1			2		
	0			5		
	0			2		
				1		
mean	1.4	1.2	1.14	2.4	1.15	0.6
max.	4	3	3	5	3	2
S.D.	1.2	1.1	1.3	1.6	0.8	0.8
No.	24	20	20	25	20	20

Table 41 . Growth experiment; increase in length(mm) ,original length 5mm.

	0061 05	0061 11 to 0061 05	0061 11	0061 05 to 0061 11	0071 98	0061 05 to 0071 98
Day 51						
	5	3	5	0	1	0
	0	3	4	0	5	2
	6	9	1	5	0	4
	6	4	1	1	5	0
	3	2	3	7	3	0
	2	6	0		2	2
	5	6	5		2	0
	6	0	2		1	1
	0	4	4		1	2
	3	4	3		2	1
	5	4	2		2	0
	3	4	1		3	2
	4		0		1	1
	1		2		1	3
			2		4	1
			4		3	1
					2	0
					2	0
					3	1
					3	3
					2	
					3	
					2	
					4	
					2	
					3	
					3	
					1	
					0	
mean	3	4.1	2.4	2.6	2.3	1.3
max.	6	9	5	7	5	4
SD.	2.1	2.3	1.6	3.2	1.3	1.2

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